PHYSIOLOGICAL DISORDERS OF TROPICAL AND SUBTROPICAL FRUITS WITH PARTICULAR REFERENCE TO CHILLING INJURY, OLEOCELLOSIS, AND STYLAR END BREAKDOWN

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KEY TO SYMBOLS AND ABBREVIATIONS

ADP	adenosine diphosphate
ATP	adenosine triphosphate
ca.	about (*circa*)
CA	controlled atmosphere
CES	Citrus Experiment Station, Lake Alfred
concn	concentration
CO_2	carbon dioxide
DNP	dinitrophenol
DPA	diphenylamine
DPD	diffusion pressure deficit
DW	dry weight
f.b.	field box
FW	fresh weight
g	gram(s)
GA	gibberellic acid
HMPA	hexamethyl phosphoramide
IRFL	Indian River Field Laboratory, Ft. Pierce
K	electrolytic conductivity, micromhos
Ki	kinetin (6-furfurylamino purine)
kg	kilogram(s)
lbs	pounds
mg	milligram(s)
mμ	millimicron

NAD	nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
O_2	oxygen
ppm	parts per million
Q_{10}	temperature coefficient
R.H.	relative humidity
RORP	rind oil release pressure
RQ	respiratory quotient
SEB	stylar-end breakdown
SES	Sub-Tropical Experiment Station, Homestead
STD	standard
Temp	temperature
Tris	tris (hydroxymethyl) amino methane
µl	microliter(s)
#	number

INTRODUCTION

Many fruits, particularly those of tropical and subtropical origin, are injured on exposure to non-freezing temperatures below about 45 F (7.4 C), either before or after harvest. This physiological response is referred to as "chilling injury". Most of the impetus for the study of chilling has been attributable to economic problems encountered in handling and marketing of these products. Although much is known concerning temperature tolerances, visual symptoms of injury, time-temperature responses and similar empirical observations, the basic physiological mechanism remains obscure. An investigation of the nature of the physiological and biological changes involved in chilling injury is reported here, together with observations of factors controlling these changes and possible explanations as to the mechanism involved.

Previously reported physiological studies on chilling injury utilized plant parts as test materials. Except in a few cases, roots, leaves, seeds, and other plant organs, possibly varying in their responses, have been compared. Such an approach assumes a common mechanism of chilling injury among all plant organs. Although evidence for such a common mechanism has not been proved, the concept is convenient for the present study and will be used unless

evidence is found to the contrary. Consistency of effect among species was investigated by limiting this study to several types of fruits thus eliminating differences resulting from variability inherent among plant organs.

Various fruit blemishes not necessarily related to chilling, such as oleocellosis, stylar-end breakdown, abnormal rind color, and decay, were noted before or during storage. Such observations were peripheral to the study of chilling injury, but some of them were studied extensively either because of their importance as an injury per se or because their occurrence complicated the primary investigation.

The present work was designed to investigate factors involved in the sensitivity to chilling injury and other forms of rind breakdown in subtropical and tropical fruits, to explain the fundamental process or processes associated with such disorders, and finally, to devise ways by which these physiological problems could be prevented or ameliorated.

REVIEW OF LITERATURE

Chilling Injury

In plants, chilling injury often results after exposures at several degrees above 32 F (12, 13, 166, 167, 181). A similar phenomenon has been manifested by animals and lower forms as well (69, 124). The importance of chilling injury is that it constitutes a major obstacle in marketing and distribution of tropical fruits.

Low temperature injury to warm season plants was reported by Bierkander as early as 1778. His account, as cited by Molisch (124), reported that Cucumis sativus, Solanum tuberosum, Cucumis melo, Portulaca oleracea, Impatiens balsamina, and other plants were killed by exposure to temperatures 2 to 4 F above freezing.

Among fruits, chilling injury is responsible for large economic losses during storage and shipping especially when the voyage is unduly extended. The problem becomes particularly acute in mixed shiploads of fruits having different optimum storage temperatures. Because normal refrigeration can not be used, most tropical fruits never get into international commerce unless their volume justifies special ships and storages as in the case of bananas. A list of fruits susceptible to chilling together with recommended conditions of storage is presented in Table 1.

TABLE 1. Recommended storage conditions in fruits sensitive to chilling injury.

Fruits	Temperature (F)	Relative Humidity (%)	Length of storage period (days)	Reference
Avocados				
Fuerte	45	85-90	28	Overholser (137)
Pollock and Trapp	42	85-90	21	Lynch and Stahl (111)
Lula and Taylor	37-42	85-90	28	Lynch and Stahl (111)
West Indian (except Pollock and Trapp)	55	85-90	15	Wardlaw (189; Wardlaw and Leonard (191)
Bananas				
Green fruit	62-70	90-95		Banana Ripening Manual (4)
Ripe fruit	56-60	85-90	7-10	Wardlaw et al. (194)
Beans				
Green or snap	45-50	85-90	8-10	Rose et al. (153)
Cucumbers	45-50	85-90	28-42	Rose et al. (153)
Eggplants	45-50	85-90	10	Rose et al. (153)
Grapefruit	45-55	85-90	42-56	Stahl and Camp (175) Brooks and McCulloch (30)
Lemons	55-58	85-90	30-120	Hendrickson (82) Brooks and McCulloch (31)
Limes	45-48	85-90	42-56	Rose et al. (153)

TABLE 1. Continued

Fruits	Temperature (F)	Relative Humidity (%)	Length of storage period (days)	References
Melons				
Watermelon	36-40	75-85	14-21	Rose et al. (153)
Honey dew	36-38	75-85	14-28	Rose et al. (153)
Casaba	36-38	75-85	28-42	Rose et al. (153)
Olives	45-50	85-90	28-42	Pentzer and Asbury (142)
Pineapples				
Mature green	50-60	85-90	21-28	Wardlaw et al. (193)
Ripe	40-45	85-90	14-28	Williams (200)
Potatoes[a]	36-50	85-90	90-150	Stuart (178)
Pumpkins	50-55	70-75	60-180	Rose et al. (153)
Squash	50-55	70-85	60-180	Rose et al. (153)
Sweet Potatoes	55-60	75-85	120-180	Leuritzen (100) Thompson (180)
Tomatoes				
Ripe	40-50	85-90	7-10	Wright and Gorman (202)
Mature green	55-70	85-90	21-35	Wright et al. (203)

[a]A different type of chilling injury. It is associated with starch-sugar metabolism and is reversible.

Generalizations concerning the optimum storage temperatures for fruits of even a single species may be unsafe. Overholser (136) states that much depends upon the particular variety, the previous environment, the stage of

ripeness attained when harvested and stored, duration of the particular storage temperature, the amount of aeration and other factors. Thus, the temperature recommended for each type of fruit should not be considered exact but rather as safe limitations below which chilling injury may occur. A basic generalization is that many fruits can be stored at or slightly lower than 45 F. Important exceptions are bananas, lemons, limes, mature green pineapples, grapefruit, pumpkins, squash, sweet potatoes, mature green tomatoes, and many West Indian varieties of avocados.

Symptoms of Chilling Injury

A summary of visual symptoms of low temperature injury among chilling sensitive crops is presented below.

TABLE 2. Low temperature injury among chilling sensitive crops.

Crops	Symptoms	References
Avocado	Pitting or spotting of the skin	137, 111, 189,
	Greying or browning of the pulp either near the seed or in tissue midway between the seed and the skin	191, 96, 137, 177, 98
	Failure to soften when removed to a higher temperature	
	Off-taste in the flesh	
	Vascular strands develop a brownish appearance making them stand out from the lighter colored pulp	

TABLE 2. Continued

Crops	Symptoms	References
Banana	Appearance of water-soaked areas	5, 6, 16, 39,
	Subepidermal brown streaking	140, 147, 169,
	Clear latex	190, 192, 204,
	Loss of flavor	188
	Delayed ripening	
	Hardening of central placenta	
	Dull sooty yellow skin color	
	Gradual closing of stomata	
	Slow starch to sugar conversion	
Cucumber	Dark colored watery areas	153, 115
	Susceptibility to mold infection	
Grapefruit	Pitting of flavedo (appears first as slightly sunken spots which increase in size forming patches). Spots turn brown with age or upon exposure to warmer temperatures. Oil glands rarely elevated above the rest of depressed areas.	175, 30, 149, 81, 66, 50, 54, 40
	Fairly uniform browning and superficial scald.	
Lemon	Pitting of flavedo turning brown with age	153, 31, 10, 66, 61
	Peel surface slow in losing color, oil glands darker than surrounding areas (petaca)	
	Reddish brown, shallow and more diffused pits but erratic in occurrence (red blotch)	
	Browning of membrane or carpellary walls between the segments (membranous stain)	

TABLE 2. Continued

Crops	Symptoms	References
Lime	Pitting of peel tissue, appearing as brown sunken spots which may coalesce to form irregular-shaped patches or bands	153, 62, 68, 198
Water-melon	Watery discoloration of the rind	153
	Tendancy to become pitted or dented	
	Objectionable flavor	
	Susceptibility to fungal attack	
Olive	Flesh of green fresh olives becomes brown beginning around the seed end at the stem end. Ripe fresh olives develop more browning than green ones	142, 153
Papaya	Water-soaking of flesh	110, 91, 92
	Failure to hydrolyze sucrose to reducing sugar	120
Pineapple	Green spotting	193, 200, 153
	Failure to develop good flavor in the flesh	
	Slow ripening	
	Susceptibility to black rot after removal to non-chilling temperature	
Potato	Undesirable sweetness develops	100, 179, 97
	Darken when cooked	
Sweet potato	Rapid browning when cut	153, 123, 108,
	Increased leakage of potassium	7, 8, 160, 9
	Decreased water absorption	

TABLE 2. Continued

Crops	Symptoms	References
	Reduced capacity to inactivate IAA	
Tomato	Failure to develop red color	202, 203, 41.
	Susceptibility to alternaria rot	14, 47, 56
	Small white pockets in skin of green tomatoes, usually near the blossom end	150, 21, 26, 196

Visual manifestations of chilling injury vary among fruits. However, pitting seems to occur in at least 60% of the fruits listed above. Water soaking and failure to ripen properly are more evident in fruits with relatively thinner or softer rind (e.g. tomatoes, cucumbers, and papayas).

Respiration Response

Symptoms of chilling injury have been linked to respiratory changes taking place in the fruit (168). One such change is an interference with normal respiratory activity. For example, Leonard and Wardlaw (103) observed that low temperature delayed the onset of ripening and had marked effects on respiration rate in bananas. Moreover, Gane (67) found that temperatures below 12.5 C (54.5 F) caused a depression of respiratory activity in bananas which was at first reversible but irreversible effects soon set in, almost completely arresting respiration.

Eaks and Morris (63, 64) evaluated chilling injury of cucumbers by the respiratory responses to chilling and non-

chilling temperatures. Cucumbers stored at 5 temperatures from 55 to 86 F all produced approximately 20 mg CO_2 per kg of fresh weight during their entire storage life. Cucumbers held at chilling temperatures produced the following amounts of CO_2: 50 F - 18 g; 41 F - 6 g; 32 F - 3 g. Cucumbers exposed to chilling temperatures and transferred to 77 F produced less than 20 gm CO_2, the amount depending upon the severity of chilling prior to non-chilling temperatures. The rate of CO_2 production decreased with duration of storage, whereas at 50 F and below, the rate increased with time to a plateau and then decreased. The increase in respiration occurred at the same time at the development of chilling injury as measured by the degree of surface pitting and the decline occurred as the tissue dried. The storage life of cucumbers at non-chilling temperatures increased from 16 days at 86 F to 62 days at 55 F. Lower temperatures reduced storage life instead of increasing it.

Factors Affecting Chilling Injury

Several factors influence the incidence of chilling injury. In bananas, chilling injury may occur even in the normal range of temperatures used in handling, depending upon the variety, maturity, grade, and length of storage of fruit. 'Gros Michel' is relatively more resistant than 'Lacatan' (190, 194, 204). Ripe fruit, regardless of variety, is reportedly less sensitive than green bananas (4). According to Segars (164), heavier (e.g. more mature) grades of 'Golden Beauty' ('Lacatan') banana are more susceptible

than lower grades whether ripe or green. Segars (164) also noted that evidence of chilling still appeared after 16 days of storage, even if the lower grades were held at the recommended temperature of 13.5 C.

Morris and Platenius (125) found that high relative humidity markedly delayed the severity of chilling injury as evidenced by pitting in cucumber and pepper.

Kidd and West (95) found that apples grown in England are more susceptible to chilling injury during their respiratory climacteric than either pre- or postclimacteric phase.

Intermediate temperatures, in some instances, give greater chilling injury than do either higher or lower temperatures. Plums stored for 25 days at 37 F showed greater injury than those at either 31 or 40 F (51). Pitting on 'Marsh' grapefruit is rarely found after 4 to 6 weeks' storage at 32 or at 50 F but intermediate temperatures frequently cause severe pitting (30, 78, 199). However, on removal to room temperatures, severe pitting may develop particularly after 32 F storage. The incidence of woolliness in peaches is much greater at 37 F than at either 31 or 45 F (52).

The greater injuries noted at the intermediate temperatures are possibly restricted to a specific time period (170,48, 182). The difference may be the result of more rapid appearance of limited injuries. After long storage periods, injuries become more nearly inversely proportional to the

temperature because the low temperature results in the slow development of injuries of a more extensive and serious nature.

Maturity of tomatoes affects the incidence of chilling injuries. McCollock (118) found that pink tomatoes in cartons could be precooled from 93 F to 45-50 F in 21 hours by circulating 32 F air. When held for 3 days at 45 F, the tomatoes had 40 to 70% red color. If held 3 days at 55 F. they were 63 to 75% colored. Thus, it is evident that temperature must be controlled rather accurately to retard ripening. There is some evidence that pink and ripe tomatoes can be stored successfully at lower temperatures than those used for mature green tomatoes. Scott and Mawes (163) showed that pink tomatoes could be stored at 32 F for 6 days and ripening then completed at 72 F with no evidence of chilling injury. Storage for 12 days at 32 F before the ripening was completed, produced off-flavor. These findings approximate those of McCollock (118) for mature green tomatoes wherein 3 to 5 days at 32 F was followed by normal ripening on removal to higher temperatures.

Cook et al. (46) reported that firm ripe tomatoes could be stored for 42 days at 32 F with a decay loss of only 3%. Ripe tomatoes were edible, had a good appearance, but had softened. These results are striking. It appears that chilling injury develops much slower, or not at all, in ripe tomatoes, and that once fully ripened, they can be handled at temperatures as low as 32 F to prevent over-ripening.

Theories on Mechanisms of Chilling Injury

The physico-chemical changes involved in the mechanism of chilling injury may be listed as follows:

1. Chemical changes,

2. Changes in the relative velocities of interrelated chemical reactions,

3. Accumulation of toxic products,

4. Abnormal respiratory behavior,

5. Role of lipids and fatty acids in chilling injury,

6. Altered membrane permeability.

A causal role for any of these mechanisms has not been proved. Few have been disproved and little evidence exists for or against others. It has been pointed out that since the above changes are complex, none is likely to be the primary cause of chilling (12, 114, 170). Also, the fact that there are at least several distinct types of injury, the symptoms observed may be the result of a complex etiology. Luyet and Gehenio (114) included cases of injury by rapid cooling. They suggested that the mechanism of injury due to rapid chilling apparently differed from that due to slow chilling, which latter probably resulted from a disturbance in the interplay of physiological functions.

However, many of the mechanisms listed above could be concurrently involved.

Chemical changes. Miller and Schomer (122) conducted biochemical studies using lemons stored at chilling and non-chilling temperatures. The peel was analyzed for sugar,

glucosides, acids, and reductase activity before, during, and after storage. Sugar content of the peel during storage did not relate to any abnormality observed in the metabolism of the fruit at all temperatures studied. No additional information on physiological disturbances was obtained from the acid and glucoside values. Reductase activity of the peel, however, as measured by the reduction of potassium permanganate solution, was consistently lower for samples stored at 32, 36, and 40 F than for those stored at 50 and 60 F. The first 3 temperatures are known to be most conducive to pitting of lemons (122). Apparently, according to Miller and Schomer (122), some substances in the peel have been oxidized more rapidly at these lower temperatures and were therefore not oxidized by the potassium permanganate solutions. The fact that sometimes the lesions are dark in color, also suggest the action of oxidases.

Changes in the relative velocity of interrelated chemical reactions. A somewhat more complex mechanism of chilling was proposed by Van der Plank and Davis (183). They considered fruit at the time of storage to have an inherent primary susceptibility which predetermines a transition temperature above which fruit will remain healthy and below which it will be injured. The transition temperature does not necessarily remain fixed but may drift as the fruit weakens in storage. They termed this shift of the transition temperature during storage, secondary susceptibility. The factors that predispose a fruit to secondary susceptibility are not necessarily the

same as those which cause primary susceptibility. The amount of injury at any given time is dependent on an equilibrium factor with change in temperature. By means of these two opposing factors, Van der Plank and Davies (183) theorized that greater injury at higher temperatures is simply a more rapid manifestation although greater injury was eventually noted at the lower temperatures.

Accumulation of toxic products. Plank (144) has offered a somewhat more simplified explanation of the chilling mechanism. He assumed that 2 main types of reactions are involved in the cells, 1 leading to the accumulation of toxin and the other to its removal. By selecting values for the temperature coefficient used in his equations, he was able to show the critical temperature at which the production and removal of toxin are in equilibrium and below which cell toxin would accumulate, causing chilling injury.

The localization of chilling injury was studied by Eake and Morris (64) by exposing one-half of intact cucumbers at 35 and the other half at 55 F. When the cucumbers were transferred to 77 after 8 days, the chilled ends appeared slightly fresher than the non-chilled ends. However, severe pitting developed on the chilled ends after 3 days and decay after 4 days. Decay failed to develop on the non-chilled portions after 8 days. If a toxic substance was responsible for the injury, it was not translocated or it was destroyed in the warmer end.

Smith (170) provided further evidence for the theory of

toxic material in his work with 'Victoria' plums. Severe injury was obtained after storage at 31 F for 5 weeks. When the storage period was interrupted after 15 to 20 days by a 2-day period at 65 F and the plums were further exposed to 31 F for 15 to 20 days, there was little or no injury. Brooks, Cooley, and Fisher (32) found a beneficial effect of subjecting apples to brief warming periods which gave almost complete control of scald in susceptible varieties. In this case, it was suggested that the toxic substance was a volatile accumulated at cold storage temperature and expelled at warm temperature. This led to the concept of dual temperature treatment. Best control of woolliness in peaches consisted of subjecting fruit to 31 F for 5 to 10 days before the remainder of the storage period at 45 or 50 F (52). This has become a standard practice in the refrigerated transport of South African plums and peaches to the United Kingdom. Again, the effectiveness of dual temperature treatments can be pictured hypothetically as resulting from accumulation of a toxic or inhibiting substance which, if accumulation has not proceeded too far, can be removed at a higher temperature. Nelson (130) proposed that the toxic material may be a fragment of a hydrolyzed glucoside and that unfavorable external conditions prevent the normal detoxification process.

Although the accumulation of sugars, organic acids, and other slight changes, have been noted in certain plant materials after a period of exposure to low temperature, chemical analyses for the major constituents have generally

failed to give any specific explanation of the mechanism of chilling injury. Lorenz (110), Miller (120), and Jones (91) all reported that hydrolysis of sucrose to reducing sugar was reduced in chilled papayas but found much more effect on respiration, as evidenced by the temperature coefficient at the lower temperatures.

Miller and Heilman (121) suggested that the destruction of ascorbic acid constitutes the first phase in the development of low temperature injury in pineapple. They proposed that interference in some specific steps in the respiratory process causes quinones to accumulate because of their failure to be converted back to phenols by ascorbic acid and that the accumulation of the quinones results in the discoloration noted in many kinds of chilled fruits. Pentzer and Heinze (143) failed to show any association between ascorbic acid concentration and chilling in tomatoes. Chilling conditions before harvest had little effect on the changes in ascorbic acid content during the storage period (121). Thus, the concept that chilling injury is a symptom of the accumulation of toxic substances has been prevalent since the early 1950's.

Abnormal respiratory behavior. It has been suggested (91, 105, 145, 56) that chilling injury results from disruption of the synchronization of the various stages in the complex respiratory sequence. High temperature coefficients (Q_{10}) at the lower temperatures, increasing respiratory rates during chilling, accelerated respiration following chilling, and altered respiratory quotients have all been proposed as indices of chilling sensitivity.

Jones (91) observed lower temperature coefficients in the chilling range for papaya, but most investigators (76, 131, 145) have found higher Q_{10} values in this range. Gore (71), Platanius (145), and James (87) demonstrated that high Q_{10} values could be obtained for both sensitive and relatively chilling-resistant plants and therefore this effect should not be considered causal or characteristic of chilling sensitivity.

Increased respiration during chilling has been reported for sweet potatoes (107), tomatoes (105), and cucumbers (58). None of the above authors theorized as to the mechanism responsible for the increase in respiration observed during early stages of chilling. Increased anabolism resulting in a more rapid turnover of ATP and uncoupling of respiration from oxidative phosphorylation could result in a higher respiratory rates and may occur in chilling.

Accelerated rates of respiration immediately following transfer from a chilling to a non-chilling temperature have been recognized for some time. Lewis (105) observed that tomatoes exposed to increasing periods of chilling showed an abnormal increase in carbon dioxide production following transfer to warmer temperatures. Similar results have been obtained with potatoes (1, 84, 47), apples (36, 99), citrus (60), and asparagus (126). This change-in-temperature effect was thought by some workers to result from the decreased solubility of carbon dioxide at higher temperatures. However, Lewis (105) pointed out that the amount of carbon dioxide

given off was much too large to be accounted for in this manner. Accumulation of sugars at chilling temperature could result in increased respiration following transfer to non-chilling temperatures. However, Appleman and Smith (1) observed that sugar accumulation did not affect respiratory rate at other temperatures. James (87) suggested it is possible that accumulation of organic acids, instead of sugars, in some tissues might account for the respiratory stimulation upon transfer to warmer temperatures. Attempts to correlate the increased respiratory activity following chilling with biochemical changes should await proof that the response is limited to chilling sensitive plants.

The respiratory quotient (RQ) of cucumbers (58, 115) was initially below unity at chilling temperatures, but increased with time and rose above unity after 7 days of chilling. The increase in RQ with time at chilling temperature may be associated with degenerative changes. Beevers (11) suggested that the change in respiratory quotients at chilling temperatures might reflect on the tendency of the fruit to accumulate organic acids rather than its sensitivity to chilling. This change should be considered therefore as a general response of the plant to low temperature and not a characteristic of chilling sensitivity.

Role of lipids and fatty acids in chilling injury. The role of fatty acids in chilling injury stems from their importance as major constituents of membranes, changes in viscosity of lipids with temperature, and the theory that

plants of tropical origin tend to have more highly saturated fats than plants of the temperate regions (105, 165, 45, 57, 187, 109, 141, 88, 11, 147).

Lewis (105) proposed that since cell membranes are composed of an ordered arrangement of lipid and protein molecules, chilling injury may be the result of a change in membrane permeability.

The change of some lipids from liquid to solid state at the chilling range suggests another possible relationship. This change in the viscosity of the lipids might make the cytoplasm rigid (165). Lewis (105), Cook (45), Doi (57), and Loewy (109) observed that protoplasmic streaming ceased in cells of chilling-sensitive plants at low temperature. Lewis proposed that chilling injury and cessation of streaming might be separate symptoms of a basic disorder induced by temperature in the chilling range. Alternatively, he suggested that cessation of streaming might induce anaerobic respiration, leading to chilling injury and cell death.

Plants of tropical and subtropical origin have been generally assumed to contain fatty acids that are more highly saturated than in species growing in cold regions (141, 88, 119, 101). This assumption led to the proposal that the less saturated fatty acids of temperate plants would presumably remain liquid at temperatures in the chilling range. On the other hand, the more saturated fatty acids of sensitive tropical and subtropical species may solidify at chilling temperatures, causing metabolic abnormalities and marked changes in

permeability. The basic premise that more saturated fats are found in tropical plants than those in temperate or cold regions is dubious. Primarily, this premise is based on fatty acid analysis of specialized tissues such as seeds that contain commercially exploitable amounts of fats. When chilling-sensitive and non-sensitive whole plants were used, no consistent differences in the type of fatty acids or their degree of saturation were found (197). Moreover, Hilditch (83, p. 171) stated, "to argue, as has been attempted, that tropical plants tend to produce more saturated kinds of seed fats than those of cooler habitat appears to the writer to be far too sweeping, and not substantiated by the facts."

Altered membrane permeability. Changes in membrane permeability have been suggested as possible causes of chilling injury. Weber (195), for example, reported that cells of Elodea canadensis plasmolyzed in urea became deplasmolyzed after 2 minutes at 1 C as compared to 20 to 30 minutes at room temperature. He suggested that permeability to urea increased at low temperatures because of higher degree of destruction of the ectoplast or to inhibition by cold of new ectoplast formation. Seible (167) observed that calcium nitrate penetrated chilled cells more rapidly than unchilled ones in coleus, a chilling-sensitive plant.

The permeability to water of mitochondrial membranes from chilling sensitive and insensitive species was compared at several temperatures by measuring mitochondrial swelling (112). Under controlled conditions, slight differences in

permeability of mitochondria from sensitive and insensitive plants were observed, but it is not clear whether these differences resulted from a passive movement of water or whether solute movement was also involved. Jensen and Taylor (89) studied the effects of temperature on the rate of water transport through tomato and sunflower plants under conditions which would minimize the importance of the active process. Response of these species was similar. Increased resistance to flow as temperature decreased was interpreted as resulting from changes in viscosity and other physical properties of water. Using sunflower hypocotyl segments subjected to an imposed diffusion pressure deficit (DPD), Glinka and Reinhold (70) also concluded that the influence of temperature on water absorption could be satisfactorily accounted for by the known change in the viscosity of water with change in temperature.

Rates of ion leakage during or after chilling have been studied by several workers as a possible index of chilling injury. Lieberman et al. (105) reported that leakage of electrolytes from chilled potato root tissue was 5 times greater than leakage from non-chilled tissue. However, Lewis (105) detected no change in ion leakage from tomato fruit slices with previous storage temperature. Lewis (106) reported that the rate of leakage was even greater after the higher storage temperatures. He also observed that there were no consistent differences in ion leakage rates that could be related to chilling sensitivity of tissues. Thus,

although relationships between chilling sensitivity and membrane permeability have been proposed, evidence for such relationships is not conclusive.

Research covered in this review indicates that symptoms of chilling injury are undoubtedly products of a series of degenerative changes that result from exposure to chilling temperatures. However, no report so far provides definite elucidation of the specific fundamental changes. It is assumed that a causal relationship between the fundamental process affected by low temperature and the syndrome known as "chilling injury" would exist only: a) if the particular change is confined to chilling sensitive plants; b) if this change occurs very early following exposure to low temperature; and c) if typical chilling lesions could be produced by exogenous application of the isolated factor. None of these criteria has been fully satisfied.

Oleocellosis

Oleocellosis, caused by mechanical injury to the epidermal oil cells, is a serious problem of limes and lemons. Picking and handling procedures are the principal sources of damage resulting in the rupture of oil cells located in the flavedo or epicarp (37). The more turgid the fruit when handled, the more it is susceptible to this type of injury (59). Cahoon et al. (37) noted that wherever rind oil cells are ruptured on the fruit surface, it leaves a blemish which not only degreens less rapidly than the remaining portion of

the lemon but ultimately results in a russet, brown to black discoloration. Lemons having an appreciable amount of this blemished rind can not be sold on the prime fresh fruit market but are downgraded or go to processing.

Susceptibility to oleocellosis was shown by Cahoon et al. (37) and Oberbacher (132) to be closely related with rind oil release pressure (RORP). This method is based on the pounds of pressure needed to rupture the oil glands. The lower the ROMP values, the more the fruit is blemished by handling. Thus, ROMP provides a means of predicting fruit damage prior to harvest.

Various environmental factors were associated with susceptibility to oleocellosis (37, 59, 129, 132, 181). For example, Mustard (129) reported that Florida limes picked while wet from rain or dew were susceptible to oleocellosis. Eaks (59) has shown a similar problem to exist concerning fresh limes in California. Oleocellosis could be minimized by careful picking under moist soil conditions. Turrell et al. (181) in California found a similar relationship between cool, damp weather and prevalence of oleocellosis on grapefruit. Furthermore, Oberbacher (132) observed that fruit exposed to the sun has higher ROMP values than those on the shaded side of the tree or protected by foliage. The cursory observations however, are qualitative in nature and many reports did not describe definite influences of specific climatic or nonclimatic influences.

Stylar-end Breakdown

Stylar-end breakdown (SEB) is a physiological disorder described by Pratt (149) as a water-soaked area beside the tip of the stylar-end which progresses until one-third to one-half of the rind is affected. It is apparently synonymous with the "stylar-end rot" reported by many workers (44, 46, 49). Etiological studies have been limited to empirical observations of fruit characteristics and grove conditions. A common observation is that as limes become very mature, SEB can develop prior to picking (44, 46). Thus, it is not surprising for some workers to report the prevalence of SEB among large, mature fruits than small, less mature ones during storage (38, 46, 72, 176). There were also reports that SEB is worse in those picked in the morning or when wet with dew than in the afternoon (139); higher in roughly handled fruit than those carefully picked (139, 158) and aggravated by conditions of elevated temperature and humidity (44, 176, 180). A detailed characterization of the disease has not been found in the literature.

MATERIALS AND METHODS

Fruit Sources

'Persian' limes were obtained from Highlands County, near Lake Placid, at the southern end of the Central Ridge citrus district and from 2 locations, Goulds and Homestead, in the major lime producing area, Dade County. Soil in the Lake Placid area is Lakeland fine sand; that in the Goulds-Homestead area is imperfectly weathered oölitic limestone, Rockdale series. About 95% of the trees were on rough lemon stock; the rest were either own-rooted marcots or on Citrus macrophylla stock. Fruit was harvested from 3 trees each of 'Marsh' and 'Duncan' grapefruit in control lots of a former fertilizer experiment located at CES grove. The soil is Lakeland fine sand. 'Valencia' orange was included in the study as a control fruit. Fruit was obtained from the CES grove as needed. 'Lacatan' bananas, secured at dockside from the Banana Trading Company, Tampa, were 13 days from picking at Esmeraldas, Ecuador, and had been 11 days in transit at 56 F (S. Schiro, personal communication). Bunches with 14 hands were selected. Hands were numbered according to their position on the bunch and stored at 60 F prior to treatment. Off-season 'Booth 5' avocados were obtained from SES. Mature green 'Floradel' tomatoes were secured from control plots of a breeding experiment at IRPL.

Determinations of Factors Before and During Storage

This study dealt largely with 'Persian' limes· therefore methods will be discussed for this fruit, those for others being mentioned only when different. Major experiments are summarized in Table 3.

Variability in results of storage experiments can often be traced to preharvest conditions; thus, an effort was made to investigate certain environmental factors before and during harvesting which might influence subsequent chilling injury.

Data were taken on temperature, relative humidity, light intensity, wind velocity, and precipitation in the grove. Temperature, as degrees F, and relative humidity, as percentage, were recorded on a Brown hygrothermograph. Periodic readings of light intensity were made with a Weston light meter calibrated in foot candles, and of wind velocity with an Alnor velometer as miles per hour. Precipitation consisting of drizzle, showers or continuous rain was noted as it occurred during harvesting.

Preharvest, harvest, transportation, postharvest, and packinghouse operations investigated are listed in Table 4.

Histological Techniques

Samples of chilled and unchilled peel were sectioned in a rotary freezing microtome. Sections were dehydrated and stained with either Sudan IV or safranin, following procedures of Johansen(90).

TABLE 3. Summary of experiments conducted.

Experiments	Lisse	Grapefruit	Chilling injury Banana
A. Pre-harvest			
1. Fruit age	X	X	
2. Fruit location on tree	X	X	
B. Harvest			
1. Climatic factors			
a. Temperature	X	X	X
b. Relative humidity			
c. Light intensity	X	X	
d. Rainfall			
e. Wind velocity			
2. Non-climatic factors			
a. Time of day	X	X	
b. Fruit age	X	X	X
c. Fruit location on tree			
d. Position on field box			
3. Handling methods	X		
C. Transportation	X		
D. Packinghouse			
1. Kinetin, "wax" & diphenylamine	X		
2. Kinetin and gibberellic acid	X		X
E. Storage			
1. Location of hand on bunch			X
2. High temperature	X		X
3. Humidity in storage	X	X	X
4. Temperature conditioning	X	X	X
5. Partial vacuum	X	X	X
6. Controlled Atmosphere storage	X		
F. Anatomical observations	X	X	X
G. Physical investigations			
1. Respiratory activity	X	X	X
2. Permeability studies	X	X	X
3. Lipid content	X	X	
4. Accumulation of metabolites			
a. Organic acids	X	X	X
b. Metabolic inhibitors	X		X
5. Mitochondrial activity	X	X	
6. Volatiles	X	X	

PEEL INJURIES AND COLOR						
		Rind Color		Oleo-cellosis	Stylar-end Breakdown	Decay
Tomato	Avocado	Limes	Banana	Limes	Limes	Limes
		X		X	X	
				X		
		X	X	X	X	
				X		
				X		
		X		X	X	X
				X		
				X	X	X
			X	X	X	
				X		
				X		
		X		X	X	X
		X		X	X	X
		X				
		X	X			
			X			
		X				
	X					
	X		X			
X	X	X				
		X			X	X
X	X			X	X	
						X
X	X	X	X			
X	X				X	
					X	
X		X				

TABLE 4. Description of treatments from pre- to postharvest operations.

Treatment	Description	No. Fruits per replication	No. Replications
Preharvest factors			
Location on tree	Fruits about 55 g wt. from inside, outside, north, east, south, and west of tree tested for ROEP using 2 Magness-Taylor pressure testers with 3/8 inch head, 0 to 10 and 0 to 30 lb scales, respectively (37, 116).	10	5
Fruit age (size)	Fruits with diameters from 0.8 to 2.4 in. taken from south side of tree.	140	3
Harvest			
Time of day	Fruit picked morning, noon, and afternoon.	10-20	5
Very careful handling	Clipped with pickers glove padded with 0.5 in. thick polyurethane foam.	10-35	5
Normal handling	Commercial picking	10-35	5
Immediate rough handling	Clipped, pressed 6 times at 10 lbs pressure and stylar-end hit 3 times on hard corrugated fiberboard immediately after picking.	10-35	5
Delayed rough handling	Clipped, pressed 6 times at 10 lbs pressure and stylar-end hit 3 times on hard corrugated fiberboard 24 hours after picking.	10-35	5
Morning picking normal	Commercial picking	10-35	5

TABLE 4. Continued

Treatment	Description	No. Fruits per replication	No. Repli-cations
Morning picking rough	Immediate rough handling	10-35	5
Noon picking normal	Commercial picking	10-35	5
Noon picking rough	Immediate rough handling	10-35	5
Afternoon picking normal	Commercial picking	10-35	5
Afternoon picking rough	Immediate rough handling	10-35	5
Position in field box	ROAF of fruits at top, middle, and bottom of field boxes taken at 1:00 p.m.	10-15	3
Refrigeration			
Ultra-prompt	Limes placed in "Vexar" (polyethylene net) bags, wrapped with polyurethane foam sheets, and put in insulated ice chest immediately after picking.	10-35	5
Normal	Limes held at 50 F 24 hours from picking.	10-35	5
Transportation			
Experimental samples	Transported by car from grove to packinghouse in ventilated polyethylene bags.	10-35	5
Commercial operation	Samples taken from a) field boxes, b) after dumping field boxes into pallet boxes, c) on arrival at	150-250	1

TABLE 4. Continued

Treatment	Description	No. Fruits per replication	Replications
	packinghouse by tractor trailers, and d) after dumping.		
Postharvest			
Waxing	Limes either treated with "Flavorseal 93" (coumarone-indene resin in an organic solvent) on CES packinghouse line or hand-dipped in a water-emulsion citrus wax.	10-250	3
Chemical treatments	Kinetin (10-100 ppm), gibberellic acid (10-1000 ppm, and diphenylamine (500-2500 ppm) applied singly or in combination. Fruits dipped twice and air-dried after each dip.	10	3
Storage			
Temperature	Limes placed in 5 storage rooms maintained at 32, 40, 50, 60, and 70 F and 5 cabinets at 36, 55, 85, 90, and 110 F.	10-35	5
Relative humidity	Samples were held inside desiccators at 40 F and relative humidities of 50, 75, and 100%. Continuous air flow was provided.	18	3
Temperature preconditioning	Fruits held initially at 70 F with final temperature after 8 days of 40 F. In the 1st lot, temperature was reduced in 5 F steps at 1-day intervals in the 2nd, 10 F at 2-day intervals, in the 3rd, 15 F at 4-day intervals, and in the 4th, limes held for 8 days at the initial 70 F temperature and then transferred to 40 F.	15	4

TABLE 4. Continued

Treatment	Description	No. Fruits per Replication	No. Replications
Partial vacuum at chilling temperature	Samples subjected to partial vacuum of 220 mm Hg and held at 40 F (see Fig. 1).	15	3
Controlled atmosphere storage	Limes enclosed in 5-gallon glass jars sealed with metal lids having diffusion holes of from 1/32-1/8 in. diameter (158). A 5 ml sample was withdrawn through the diffusion hole for analysis on Fisher clinical gas partitioner, using HMTA and molecular sieve: Fisher No. 11-134-45 matched set for CO_2. O_2, and N_2.	10-35	5

Physiological Investigations

Respiratory Activity Determination

Respiratory activity was measured on duplicate samples of individual fruit with a Beckman IR-nondispersion CO_2 analyzer and recorded on a Sargent recorder in microamperes. Data are reported as mg CO_2 $hr^{-1}kg^{-1}FW$ (185, 186). Respiration of sections or parts of fruit was determined in a Warburg respirometer in terms of μl O_2 $hr^{-1}g^{-1}FW$.

Rind Color Measurement

Blanched (white to yellow) areas on each fruit were

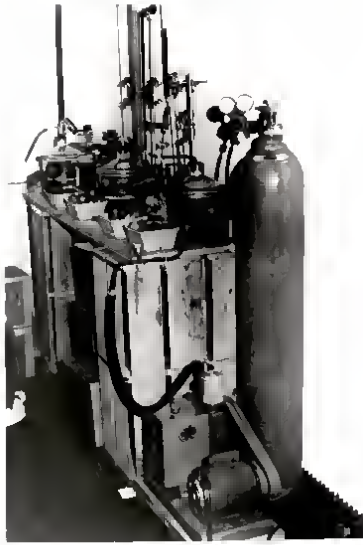

Figure 1. Experimental arrangement for subjecting fruits to partial vacuum.

marked. Fruit color of [illegible] was determined as absorbance at 675 mμ on a Bausch & Lomb Spectronic 20 [illegible].

Electrolytic Conductivity Determination

Samples were washed with soap, rinsed twice with distilled water, and then dipped in a liter of double distilled water for 24 hours. Electrolytic conductivity of the solutions less a water blank was determined with an Industrial Instruments Inc., Model RC-16B1 conductivity bridge. Data are expressed as microohos per 100 sq cm of fruit surface.

Lipid Analysis

The rapid method of lipid extraction proposed by Bligh and Dyer (28) and Borgstrom (29) as modified by Wheaton (197) was used. Data are expressed as mg lipids per g FW.

Estimation of Organic Acids

Ten ml of peel and juice vesicle extracts, representing about 1.5 g peel and 4 g juice vesicles, were boiled with 1 ml 0.1 N HCl together with 2 ml trichloroacetic acid to precipitate proteins. The filtrate was used for identification of organic acids.

Standard concentrations of individual organic acids were spotted on 8" x 24" sheets of Whatman No. 1 filter paper and on thin-layer chromatograms. The solvent mixture

employed was 1- pentanol and 5 N aqueous formic acid, prepared at least 3 hours before use by mutually saturating equal parts by volume of the two components (33). Developed chromatograms after drying were sprayed with 0.05% bromcresol green in 95% ethanol. Areas of spots were determined and plotted against concentration. Twenty µl portions of samples were similarly spotted and developed. Organic acidsin samples were identified and quantitatively estimated by comparing against concentrations of known organic acids.

Determination of Mitochondrial and Oxidative Phosphorylation Activities

Mitochondrial activity. Mitochondria were isolated from limes and grapefruit according to the methods of Vines and Oberbacher (185) and Vines and Metcalf (184). Mitochondrial activity was determined in terms of µl O_2 per hour per g fresh weight in a Warburg respirometer. The reaction mixture in Warburg flasks contained mitochondria and the following co-factors: 12.5 µmole organic acid, 30 µmoles potassium phosphate, 12.5 µmoles $MgSO_4$, 10 µmoles adenosine diphosphate, 0.5 mg cytochrome C, 50 µmoles glucose, 0.5 mg hexokinase, 0.2 mg thiamine pyrophosphate, and 0.1 mg coenzyme A. After a 10 min equilibration period, the reaction was initiated by tipping the vessel containing the organic acid in the side arm. The reaction was terminated after incubation for an hour.

Oxidative phosphorylation activity. Phosphate was analyzed by the procedure of Taussky and Shorr (179). The

phosphate content of the reaction mixture was compared to a blank for calculation of the P/O ratio.

Reaction products were identified and estimated by paper chromatography.

Identification of Fruit Volatiles

Chilled and unchilled fruits were placed in a 400 ml beaker and covered with a double layered thin film Saran wrap (polyvinylidene chloride). Samples of emanations were taken by inserting a syringe through a small strip of adhesive taped over the Saran wrap. Identification of volatile compounds was made on an Ionic Research, Inc., Model 720 dual column programmed temperature gas chromatograph. Concentrations of known samples were used for quantitative estimation. Data are expressed as g per kg fresh weight of tissue.

Addendum for Grapefruit and Banana

Grapefruit

Seasonal changes. The degree of susceptibility to chilling and varietal differences were followed throughout the growing season. Five fruits were harvested from the eastern, southern, western, northern, top, and inside portions of the tree at biweekly intervals and held at 40 F. Observation on per cent of fruit surface pitted (Fig. 2) for each lot of fruit was made 6 weeks after picking.

Light exposure on the tree. Fruits were covered about

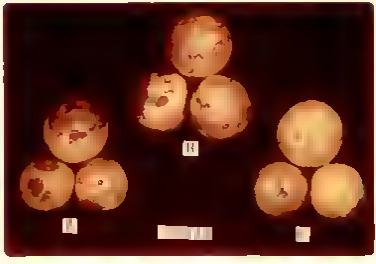

Figure 2. Different degrees of chilling injury in 'Duncan' grapefruit (A - more than 35% of fruit surface pitted; B - 15 to 25% of fruit surface pitted; and C - 5 to 14% of fruit surface pitted).

3 months before harvest with paper and plastic bags, cut open at the bottom, to study the effect of light on subsequent susceptibility to chilling. This treatment was compared with fruits covered only with transparent plastic bags. Twenty samples were distributed at the east and south sides of the tree. Fruits were stored at 40 F after harvest.

Banana.

Location of hand on the bunch. Variations in chilling injury were studied with respect to position of fruits on the bunch. Bunches with only 14 hands were selected and each hand limited to 16 fingers. Hands and fingers were detached and numbered. Fruits were held at 60 F for 4 days - at this time, blossom-end fruits were still at stage 1 (4) and stem-end fruits were already at stage 2 (4) - before they were transferred to 40 F and observed after 6 days. Chilling injury was recorded as per cent of finger pitted (or with smoky appearance) at various locations of hands on the bunch.

RESULTS AND DISCUSSIONS

Storage disorders in many fruits result when subjected to non-freezing temperatures below about 40 F. This physiological disorder is referred to as chilling injury. Factors involved in the sensitivity to chilling injury, the basic process or processes associated with such disorder, and measures to prevent or ameliorate this type of rind breakdown, were investigated. Fruit blemishes other than chilling injury, such as stylar-end breakdown, abnormal rind color, oleocellosis, and decay, were noted before or during storage. Some of them were studied extensively because of their economic importance as an injury per se.

Many of the preharvest factors studied did not affect chilling injury of limes to any extent. Consequently, the preharvest work was mainly on oleocellosis and stylar-end breakdown.

Oleocellosis and Stylar-end Breakdown

Oleocellosis

Oleocellosis is directly related to turgidity of cells on fruit surfaces as evidenced by low RORP values for turgid fruit - thus it is logical to study the factors effecting water relations of the fruit. Various climatic and non-

climatic influences were investigated, determined and correlated with RORP values.

Climatic Factors

A rise in temperature from 65 to 92 F, with a corresponding fall in relative humidity from 80 to 32% starting at 7:00 a.m. up to 2:00 p.m., increased RORP by about 18 pounds (Fig. 3). Low light intensity, about 100 foot candles, on the north side of lime trees within a row in the grove (Fig. 4) did not markedly lower RORP of fruit on that side (Fig. 5). Apparently, ambient temperature and relative humidity affected RORP values more than did light intensity per se. It was further observed that a drizzle during a cloudy day would result in a decrease in RORP of about 2 lb (Fig. 5). Also, when rain was continuous and heavy as on June 9 (Table 5), RORP values were invariably low. Wind in the grove up to 3.4 miles per hour had no effect on RORP (139). Of the climatic factors studied, only temperature and relative humidity were critical. These exerted marked and coherent effects on non-climatic influences subsequently studied.

Non-climatic Influences

Time of day. On a bright, sunny day, RORP values of limes increased steadily from about 3 to 21 lb through the morning and mid-day with a peak at about 2:00 p.m. (Fig. 3) On a cloudy day, however, the increase in RORP was much

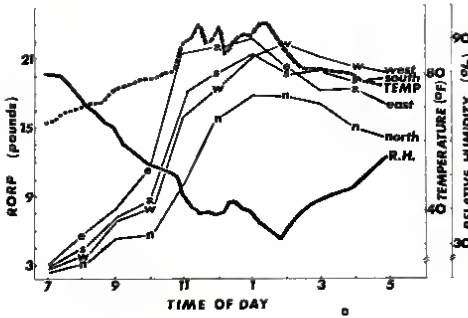

Figure 3. Diurnal variations in rind oil release pressure (RORF) of 'Persian' limes as affected by microclimate (Temperature= TEMP and relative humidity = R.H.) and position of fruit on the tree. A 0 to 30 lb scale Magness-Taylor pressure tester with a 3/8 inch head was used.

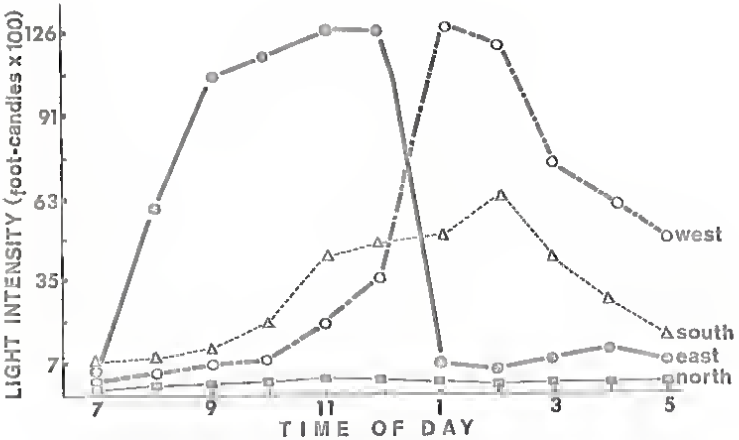

Figure 4. Hourly variation in light intensity during a sunny day at various locations around 'Persian' lime trees.

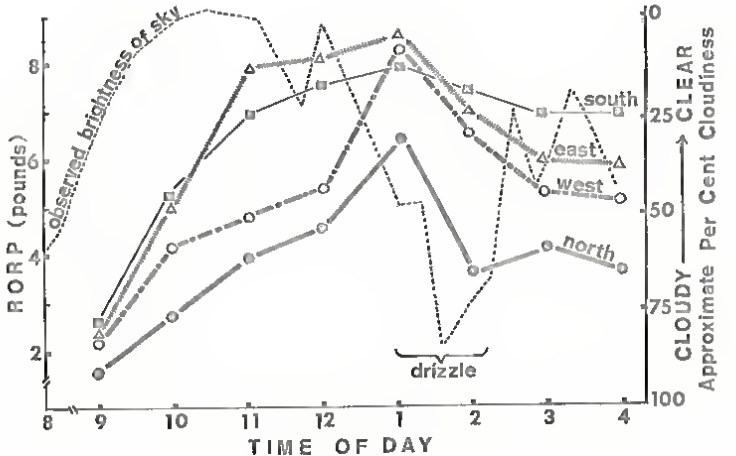

Figure 5. Hourly variation in rind oil release pressure (RORP) of 'Persian' limes at various locations around the tree during a cloudy day. 0 to 10 lb scale Magness-Taylor pressure tester with a 3/8 inch head was used.

TABLE 5. Diurnal variation in rind oil release pressure (RORP) of 'Persian' limes picked at approximately biweekly intervals.

Picking date	7:00-8:00 a.m.	12:00-1:00 p.m.	5:00-6:00 p.m.
May 11, 1966	5.3	7.7	9.0
May 25, 1966	3.1	9.2	6.4
June 9, 1966	1.8	3.2	3.0
June 22, 1966	2.0	10.0	10.0
July 13, 1966	2.9	20.5	16.8

clower and was less than 10 lb at mid-day (Fig. 5). Risk of oleocellosis increases as RORF values decrease in late afternoon. Conditions in the tree at any particular time of day could delay the increase in RORF. Fruits clipped at about 10:00 a m and hung at the same position in the tree had higher RORF values during the rest of the day than adjacent attached fruit (Fig. 6).

Fruit age. RORF values of limes were related to fruit diameter. The curve of RORF plotted against diameter (Fig. 7) was sigmoidal, with a critical range at about 2 inches. Young fruits with rough surfaces were much more susceptible to oil gland rupture than large mature fruits with smooth surfaces.

Location of fruit on tree. RORF of fruit on the east side of trees was highest from 7:00 a m , 3 lbs; to 1:00 p m , 22.5 lbs, but dropped to 18 lbs at 5:00 p m (Fig. 3). Limes from the north side gave the lowest values throughout the day. A few points may be deduced from these observations. During conditions which cause low RORF, extreme care should be taken in picking the north side of the tree. It is advisable to start picking only on the east and south sides of trees at about 10:30 a m , pick the north side at noon, and then transfer to the west side early in the afternoon.

Position of fruit in field box. Variations in RORF among fruits in field boxes were observed after about 6 hours from picking. Fruits in the top of a field box had higher RORF values, exceeding 10 lbs at 2:00 p m than fruits in the middle or at the bottom of the box (Fig. 6). RORF values from the latter two locations attained only about 8 lb.

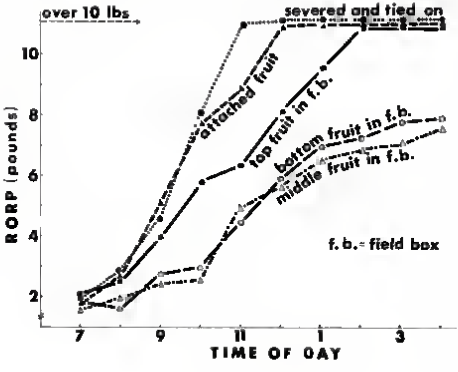

Figure 6. Hourly variation in rind oil release pressure (RORP) from the trees to the field box. A 0 to 10 lb scale Magness-Taylor pressure tester with a 3/8 inch head was used.

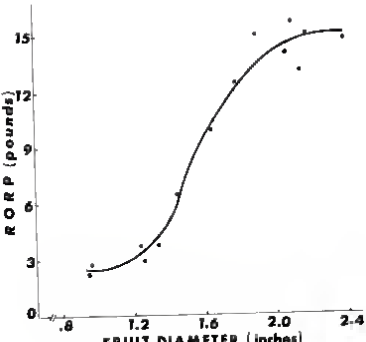

Figure 7. Relationship between size of fruit and rind oil release pressure (RORP). Each point represents mean of 10 fruits. A 0 to 10 lb scale Magness-Taylor pressure tester with a 3/8 inch head was used.

Handling Methods

Incidence of oleocellosis in limes was most severe with fruit picked in the morning, 22.8%, than with those picked at noon, 8.6%, or in the afternoon, 10.2% (Table 6). When rough handling was done at these times, oleocellosis increased further and was most severe again in the morning picked fruit. Moreover, rind oil spotting was more, 60.9%, when fruit was roughly handled immediately after picking than when rough handling was delayed for 24 hours, 27.7%. A similar relationship between oleocellosis of limes and handling methods was observed by Eaks (59) in California. When Eaks used 3 picking treatments, clip-picked, snapped gently, and snapped roughly, oleocellosis in limes was minimized by careful picking even under moist soil conditions.

Transportation

As mentioned above, oleocellosis was found to increase with deliberate rough handling. These tests under artificial conditions were compared with commercial conditions. Single-box samples were taken from successive stages in a commercial harvesting operation. Each operation resulted in an appreciable increase in oleocellosis among samples held for observation (Table 7). Samples taken directly from field boxes had 18.3% rind oil spotting, and this value almost doubled to 35.5% after dumping in the packinghouse. Hence, results obtained under commercial handling practices corroborated those found experimentally.

TABLE 6. Effect of handling method, time of day when picked, and holding temperature on oleocellosis of 'Persian' limes.

Treatment[a]		Oleocellosis after 1 week (%)
Handling method[b]		
Very careful		5.9
Normal		6.6
Immediate rough		60.9
Rough after 24 hours		27.7
Time of day when picked[b]		
Morning	Normal	22.8
	Rough	88.5
Noon	Normal	8.6
	Rough	60.9
Afternoon	Normal	10.2
	Rough	36.6
Holding temperature		
Normal handling	32 F	8.0
	40 F	10.2
	50 F	10.2
	60 F	12.0
	70 F	10.9

[a]Five replications of 10-35 fruits each.

[b]Held at 50 F.

TABLE 7. Percentages of oleocellosis in samples of 'Persian' limes from a commercial harvesting operation after holding at 50 F for 3 weeks.[a]

Sampling point	Oleocellosis (%)	Increment
From field boxes	18.3	--
After dumping field boxes into pallet boxes	25.2	+6.9
On arrival at packinghouse	29.6	+4.4
After dumping	35.5	+5.9

[a]About 280 fruits per sample, single test.

Only a few of the various environmental components which might effect oleocellosis were considered here but they serve to establish certain principles regarding the behavior of fruits prior to storage. Rind oil spotting varied with age of fruit and location on the tree. Young lime fruits located at the north side of tree are more susceptible to oleocellosis than mature fruits from the east side, especially when picked early in the morning. Rough surfaces of young fruit were conducive to oil gland rupture. Conditions, such as low temperature, high relative humidity, rainfall, cloudiness, leaf shading, and fruit overlapping in field boxes, which maintain turgidity of fruit also maintain susceptibility of the fruit to oleocellosis. Thus, turgid limes must be handled gently at all times.

Stylar-end Breakdown

According to Conover (44), a survey among various groves showed that the extent of injury as a result of SEB may range from 6 to 60% in 'Persian' limes (44). Investigations on handling methods and induction and development of SEB were therefore initiated because of the economic importance of this malady.

Handling Methods

When samples were taken from successive stages in a commercial harvesting operation, the increase in SEB after dumping field boxes into pallet boxes was 5.4% for a total of

6.4% (Table 8). No increase was observed on arrival at the packinghouse but SEB increased another 3.0% after dumping. Immediate refrigeration did not affect incidence of SEB, rind color, percentage decay, or respiratory activity of limes (Table 9).

Induction and Development

The origin and development of SEB is not known. In the present work, 2 forms of SEB in limes were apparent, one that which is initiated by mechanical injury and another that occurs as the fruit matures.

Mechanical injury. Striking the apex of lime fruit could drastically increase SEB. Such treatment immediately after picking produced 5 to 6 times the amount of SEB for limes picked in the morning as compared with those picked in the afternoon. No further effect was observed when rough handling was delayed for 24 hours after picking (Table 10). These effects were demonstrated to result from localized stresses around the stylar-end tip (Fig. 8-A to 8-D). When the fruit was flaccid (Fig. 8-C), pressure on the tip merely depressed the entire end of the fruit with no apparent localization of stress. When the lime was turgid (Fig. 8-D), pressure on the tip forced the apex downwards, exerting a marked shearing action in the annulus of thin peel tissue around the tip. A longitudinal section through the fruit showed that moisture was exuded in the annulus when pressure was applied to the apex. Furthermore, it was seen in limes deliberately

TABLE 8. Percentages of stylar-end breakdown in samples of 'Persian' limes from a commercial harvesting operation after holding at 50 F for 3 weeks.[a]

Sampling point	Stylar-end breakdown (%)	Increment
From field boxes	1.0	--
After dumping field boxes into pallet boxes	6.4	+ 5.4
On arrival at packinghouse	6.3	- 0.1
After dumping	9.3	+ 3.0

[a]About 280 fruits per sample; single test.

TABLE 9. Effect of prompt, as compared to delayed, refrigeration on respiratory activity, color, and peel injuries of 'Persian' lime fruits.

Criteria	Immediate refrigeration (prompt)	Refrigeration after 24 hours (delayed)
Color values[a] after 4 weeks (as absorbance at 675 mμ)	1.02	1.00
Respiratory activity[b] (mg CO_2 $hr^{-1}kg^{-1}$)	7.50	7.79
Stylar-end breakdown after 4 weeks (%)	0	0
Decay after 6 weeks (%)	18.75	20.00

[a]High absorbance values indicate dark green; low values, pale green.

[b]At 60 F, subsequent to 3 weeks' storage at indicated temperatures. Increased respiration rate indicates internal injury.

TABLE 10. Effect of handling method, time of day when picked, and holding temperature on stylar-end breakdown of 'Persian' limes.

Treatment		Stylar-end breakdown after 4 weeks
		(%)
Handling method[b]		
Very careful		0
Normal		0
Immediate rough		22.5
Rough after 24 hours		25.4
Time of day when picked[b]		
Morning	Normal	0
	Rough	61.0
Noon	Normal	0
	Rough	22.5
Afternoon	Normal	0
	Rough	11.9
Holding temperature (Normal handling)		
	32 F	5.7
	40 F	1.5
	50 F	0
	60 F	0
	70 F	0

[a]Five replications of 10-35 fruits each.

[b]Held at 50 F.

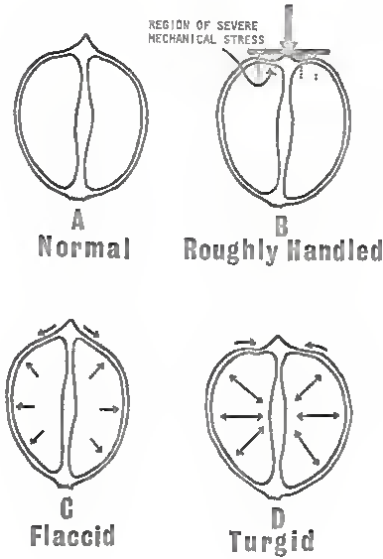

Figure 8. Influence of mechanical injury and turgidity on susceptibility of limes to stylar-end breakdown: (A - normal fruit; B - impact on the mammiform tip exerts a shearing force on a narrow region of peel tissue around the stylar-end; C - a flaccid fruit is free to absorb stress by slight changes in shape; D - a turgid fruit is in a state of stress).

damaged and then sectioned as SEB progressed that a necrotic area began at the junction of flavedo and albedo and progressed down the sides of the fruit.

Transmission. SEB could be transmitted from fruit to fruit. Twelve of 20 deliberately injured sound limes that received "caps" (Fig. 9) from SEB-affected fruit developed SEB (Table 11). Merely pricking the tips of the limes did not cause SEB. This result was substantiated when extract from SEB lesions injected into sound fruits resulted in SEB after a week (Fig. 10). These observations lead one to the hypothesis that mechanical injury causes a release of cell contents from the tissue at the junction of the flavedo and albedo and peripheral to the stylar-end. The cell contents, on being released, damage membranes of contiguous cells, thereby initiating a chain reaction of rapidly developing necrosis.

The nature of the particular constituent or characteristic of cell contents causing SEB was assessed by injecting 0.2 ml of macerated and extracted tissue from SEB lesions under the flavedo near the equatorial region of the fruit. This procedure was repeated with several solutions. Extract from SEB-affected fruit produced the most SEB, 90%, and consequent decay, followed by HCl at pH 2.0 (Table 12). These results indicate that the cause of SEB apparently is not a very complex biochemical compound.

Maturity. It is common observation that susceptibility to SEB coincides with advancing fruit maturity (2, 36, 44,

Figure 9. A simple method to demonstrate transmission of styler-end breakdown from cut styler-ends ("cams") with lesions to sound lime fruits.

TABLE 11. Transmission of stylar-end breakdown through mechanically injured styler tip (10 fruit per sample).

Treatments			August 1966[a] SEB (%)	August 1966[a] Decay[c] (%)	May 1967[b] SEB (%)	May 1967[b] Decay[c] (%)
Without caps	Not pricked		0	0	0	0
	Pricked		-	-	0	0
With caps	Caps without SEB	Not pricked	-	-	0	0
		Pricked	-	-	10	0
	Caps with SEB	Not pricked	20	10	10	10
		Pricked	60	20	80	50

[a]Observed 8 days after treatment.

[b]Observed 10 days after treatment.

[c]Severe SEB lesions soon became infected with green mold.

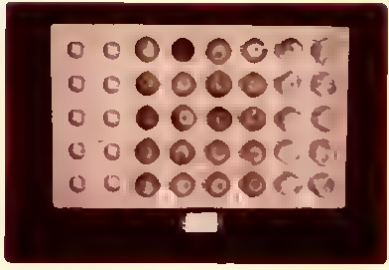

Figure 10. Transmission of stylar-end breakdown (SEB) from juice of affected fruit to sound limes. (A - injected with H_2O; B - injected with juice taken from macerated vesicles of normal fruit, C - injected with juice taken from macerated vesicles of SEB-affected fruit.) Green and blue mold was observed in lot C subsequent to occurrence of SEB.

TABLE 12. Injection of 'Persian' limes with 0.2 ml of tissue extract, hydrochloric acid, trichloroacetic acid, and a composite sample of organic acids to induce stylar-end breakdown.

Treatments	Stylar-end breakdown[a] (%)	Decay[a] (%)
Control	0	0
Water	10	0
Tissue extracts from sound fruits	45	25
Tissue extracts from SEB lesions	90	40
Hydrochloric acid = pH 2.0	75	20
= pH 6.5	20	0
Trichloroacetic acid = pH 2.0	70	0
Organic acids (composite)[b]	30	10

[a]Observed 9 days after treatment

[b]A mixture of citric acid, succinic acid, α-ketoglutaric acid, malic acid, fumaric acid, and pyruvic acid adjusted to pH 6.5 with NaOH.

146, 176). Mature fruits given a post-harvest simulated rainfall for 10 hours suffered more SEB than did the unsprayed controls. (Table 13). SEB did not develop when this experiment was repeated with small, immature limes. Apparently, a certain degree of maturity is necessary before SEB can be mechanically induced. It is possible that the variation of SEB with maturity could result from differences in composition between the stem and stylar ends. Haas and Klotz (75) and Bartholomew and Sinclair (10) have documented considerable differences in sugar content and osmotic pressure for the 2 ends of several types of citrus fruits, including lemons. If this is also true for limes, the resultant increase in turgor, together with cell membrane degeneration with advancing maturity (156, 157) would predispose the fruit stylar ends to sufficient cell rupture to initiate SEB.

Chilling Injury

Preharvest Factors

Fruit age. Chace et al. (39) showed that grapefruit harvested early in the season are more susceptible to pitting and that susceptibility to pitting decreases as the fruits become more mature. Young 'Persian' limes ranging from 20 to 22 g had 90.9% pitting after 3 weeks at 40 F (Table 14 and Fig. 11). Lighter or heavier fruits had less pitting. A marked reduction in susceptibility was observed with limes heavier than 32 g.

TABLE 13. Stylar-end breakdown as affected by turgidity of 'Persian' limes. (20 fruits per sample)

Treatment	After 15 days at 60 F	
	Stylar-end breakdown (%)	Decay (%)
Control	0	0
Fruit sprinkled with water	75	10
Fruit submerged in water	20	15

TABLE 14. Relation between age of fruit and chilling sensitivity in 'Persian' limes.

Weight of fruit (g)	Fruit surface pitted after 3 weeks at 40 °F (%)
8 - 10	57.6
15 - 17	53.8
20 - 22	90.9
25 - 27	60.0
30 - 32	18.2
40 - 43	6.7
50 - 55	5.3
100 - 120	5.5

[a] Percentage of fruit surface affected in the aggregate.

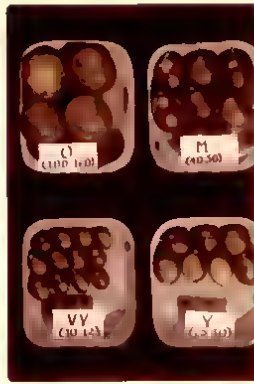

Figure 11. Variation of chilling injury in limes with age of fruit (O - old, M - mature, Y - young, VY - very young). Numbers indicate range of weight in grams. Note chilling of young fruits.

In grapefruit, however, differences in chilling susceptibility were related to both season and variety. Immature (midsummer) fruit did not develop chilling symptoms (Fig. 12). Chilling susceptibility developed as maturity approached. In 'Duncan', there was a definite peak in September (Fig. 12). In 'Marsh' however, pitting was high during the last part of July, middle of September and at the onset of October (Fig. 13).

It is difficult to explain the variations of chilling injury with age and variety. The results only confirm the observation that the history of the fruit can exert far-reaching influence on its subsequent storage behavior. The effects could result from a multitude of interrelated factors of the environment, parent materials, tree physiology, and changes in structure and composition of the fruit, especially the peel and the adjoining tissues (23, 24, 74, 79).

Location of fruit on tree. Differences in sensitivity to chilling were observed with position of fruits on the tree. Fruits picked from the top of the tree were more susceptible than those from inside both with 'Duncan' (Fig. 12) and 'Marsh' (Fig. 13) grapefruit. No difference in subsequent degree of chilling injury was observed for bagged fruit on the east or south sides of the tree (Table 15). Apparently, other climatic factors or conditions on the tree were involved. It is known that various quality factors change with position of fruit on the tree. Sites and Reitz (171, 172, 173) found that variations in the soluble solids content, vitamin C,

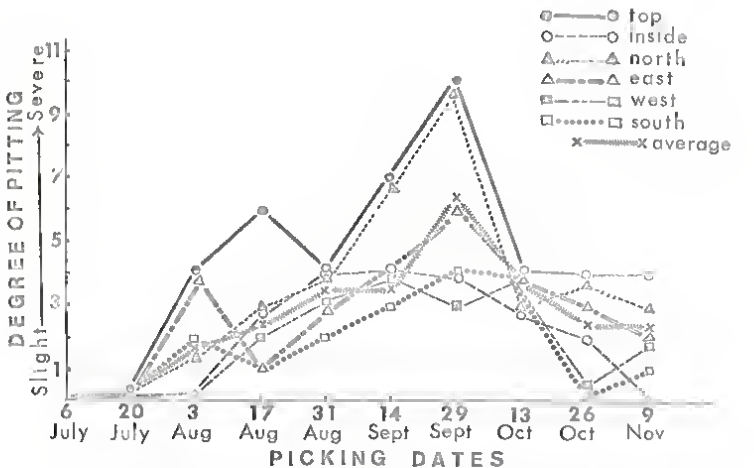

Figure 12. Variation in the degree of chilling injury on 'Duncan' grapefruit with location of fruit on the tree.

Figure 13. Variation in the degree of chilling injury on [illegible] with location of fruit on the tree.

TABLE 15. Effect of fruit shading while at the tree on subsequent degree of chilling injury (pitting) on 'Marsh' grapefruit.

Treatments	Fruit pitted after 4 weeks at 40 F			
	Sound (%)	Slight (%)	Moderate (%)	Severe (%)
Not shaded	25	40	20	15
Shaded[a]	30	35	25	10

[a]Fruits distributed at the east and south sides of the tree covered with paper and film bags cut open at the bottom. Fruits were picked after about 3 months of shading.

and titratable acidity of the juice of 'Valencia' oranges were related to direction of exposure. Chilling injury may therefore be associated with variation in fruit composition and not directly related to exposure to light.

Postharvest

Chemical treatments. A number of postharvest chemical treatments were administered to maintain healthy rinds and thereby prevent chilling injury. Kinetin (55, 127, 128, 138,152 151, 201) and gibberellic acid (35, 42, 43, 158) were used to preserve the green color of limes; whereas "wax" (Flavorseal 93) was put on to maintain turgidity. Diphenylamine (DPA), which minimizes low temperature scald in apples (32), was also applied on the presumption that it might prevent low temperature pitting among other fruits.

Aqueous solutions of 1, 10, and 100 ppm Ki and 10, 100, and 1000 ppm GA were applied to limes and bananas either singly or in combination. In limes, both chemicals progressively delayed appearance of yellow color starting at the 10 ppm Ki and 10 ppm GA concentrations (Fig. 14). However, in bananas, even the highest concentrations of 100 ppm Ki and 1000 ppm GA did not materially delay disappearance of green color (Fig. 15). Limes were therefore used in subsequent experiments.

One, 10, and 100 ppm Ki was applied in combination with 1, 2, or 3 applications of "wax". Chilling injury increased as the number of wax applications was increased (Table 16),

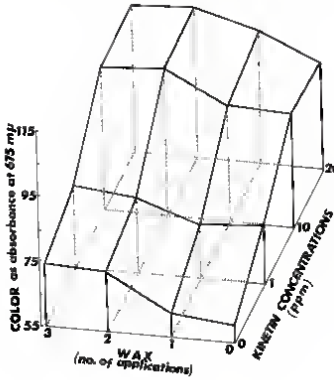

Figure 14. Effect of various levels and combinations of water-emulsion wax and kinetin on yellowing of 'Persian' limes held at 50 F for 4 weeks (high absorbance values indicate dark green, low values, pale green or yellow).

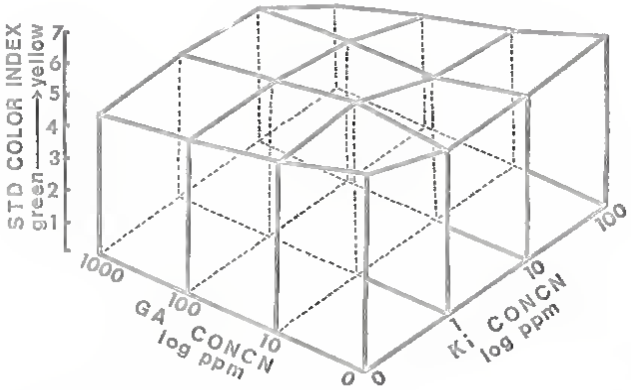

Figure 15. Effect of various levels and combinations of [illegible] (GA) and Kinetin (Ki) on yellowing of 'Lacatan' bananas.

TABLE 16. Effect of various levels and combinations of kinetin and "wax" on the incidence of chilling injury of 'Persian' limes.

Treatment		Fruit pitted after 4 weeks at 40 F[a]
Kinetin (ppm)	"Flavorseal 93" applications (no.)	(%)
0	0	43.2
1	0	45.7
10	0	39.8
100	0	33.1
0	1	51.0
1	1	63.3
10	1	48.9
100	1	47.3
0	2	76.2
1	2	61.7
10	2	80.0
100	2	68.2
0	3	83.9
1	3	78.3
10	3	88.0
100	3	89.1

[a]Percentage of fruit surface affected in the aggregate.

although retention of green color was obtained after treatment with 10 or 100 ppm Ki together with 2 or 3 applications of "wax" (Fig. 14). Chilling injury was reduced by 23.9% at the 2,500 ppm concentration (Table 17). However, when DPA was applied to limes treated with "wax" and Ki, benefits were even more evident when limes were not subsequently waxed (Fig. 16). A concentration of 10 ppm Ki combined with 250 ppm DPA produced an 80% reduction in pitting.

The results obtained here on waxing of limes do not conform with those reported by Chace et al. (39) and Davis and Smoot (54) on grapefruit, where waxing greatly reduced the development of pitting. However, Davis and Harding (53) emphasized that the type of wax emulsion formulation determined the degree of pitting to a great extent. Thus, much is yet to be learned regarding the role of waxing on the incidence of chilling injury.

Storage Conditions

No means of minimizing chilling injury are available other than maintaining temperature and exposure periods recommended for particular fruits and varieties or waxing of grapefruit (39). Two principal advantages can be expected from some means of preventing chilling injury: chilling-sensitive fruits could then be handled in the same facilities as other, less sensitive fruits and tolerance to lower storage temperatures would extend useful postharvest life. The effect of high temperature, humidity in storage, temperature preconditioning, and controlled atmosphere storage on

TABLE 17. Effect of various levels of diphenylamine combined with "wax" and kinetin on the incidence of chilling injury of 'Persian' limes.

Diphenylamine conc. ppm (combined with 10 ppm kinetin[a] and a single "wax" application)	Fruit pitted after 4 weeks at 40 F[b] (%)
Control	38.8
250	31.9
500	33.5
1000	30.6
1500	20.0
2000	21.3
2500	14.9
3000	15.6

[a]Diphenylamine and kinetin applied prior to waxing.

[b]Percentage of fruit surface affected in the aggregate.

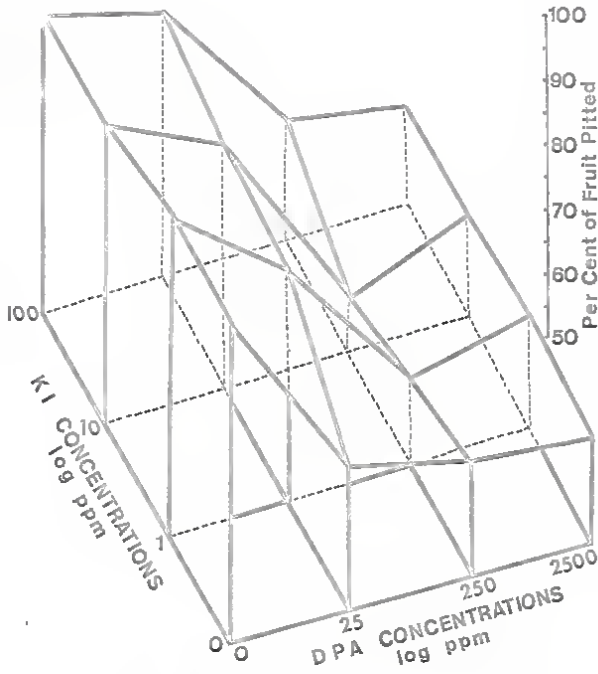

Figure 16. Effect of various levels and combinations of kinetin (ki) and diphenylamine (DPA) on the incidence of chilling injury on 'Persian' limes.

chilling injury was investigated.

High Temperature-Induced Pitting

Symptoms resembling those of chilling injury became visible in limes (Fig. 17) and bananas (Table 18) when the fruits were held at or above 90 F. Perhaps, high or low temperature pitting are basically related. This result confirms the common observation that it is necessary to shorten the time of handling from the plant to storage rooms under tropical conditions wherein field temperatures may reach exceedingly high levels (190). Pitting is likely to occur if handling under high temperatures is unduly extended.

Humidity in Storage

High humidity, 100%, ameliorated chilling injury (Table 19) and low humidity, 50%, aggravated the symptoms. The latter effect was more evident in limes, 97.6% pitting, and bananas, 83.8%, than it was with grapefruit, 22.5%. Low humidity and high temperature caused identical symptoms accounted for by partial desiccation of tissue. This similarity indicates the need for caution when the pre-history of the fruit is not known. Wardlaw (190) reported that short exposure of 'Gros Michel' bananas to 50 F produced chilling injury, but comparable fruit protected by a polyethylene bag remained unchilled. The effect was attributed to a higher temperature, 52.9 F, in the bagged fruit as compared with the unbagged. It is also possible that Wardlaw's observation on the benefits of film bags may reflect the benefits of

Figure 12. Effect of temperature on the occurrence of pitting in 'Persian' limes.

TABLE 18. Effects of temperature on color and on occurrence of pitting[a] in 'Lacatan' bananas.

Treatments	Pitting after 10 days at holding temperatures (°F)	Std. color index 3 days after removal from 40 °F[b]	Peel color[b]
90 F	30.2[c]	6	all yellow
60 F	0	4	more yellow than green
50 F	15.6	2	green-tracs of yellow
40 F	46.7	2	ditto

[a]Pitting may not necessarily be due to chilling, as in low humidity and high temperature (see text).

[b]Banana Ripening Manual (4).

[c]Percentage of fruit surface affected in the aggregate

TABLE 19. Effect of relative humidity on the occurrence of pitting in banana, lime, and grapefruit.

Fruits	Relative humidity (%)	Fruit pitted[a] (%)
'Lacatan' banana	100	3.8
	75	36.2
	50	83.8
'Persian' lime	100	5.9
	75	68.7
	50	97.6
'Duncan' grapefruit	100	10.8
	75	19.6
	50	22.5

[a]Observed after 2 weeks for banana, 4 weeks for lime, and 5 weeks for grapefruit, all at 40 F. Percentage of fruit surface affected in the aggregate.

increased humidity rather than the minor temperature difference reported.

Temperature Preconditioning

Climacteric-type fruits, such as bananas and avocados, responded to temperature conditioning but non-climacteric-type fruits, citrus, did not. Gradually lowering the temperature in 5 F stages reduced pitting on bananas from 90.6 to 8.9% and on avocados from 30.0 to 1.7%, but no effects were observed on limes or grapefruit (Tables 20, 21B, 22). Thus, the effect of gradually lowering the temperature prior to storage was apparently related to the type of postharvest metabolism involved. Lowering the temperature in 5 F stages reduced the incidence of chilling injury in bananas more than did holding at 70 F for 72 hours prior to 40 F storage (Table 21A). Also, fruits at or near the distal end were more susceptible to pitting than were those at the stem end. Physiological ages of fruit in the four treatments varied. The lot acclimatized in 5 F stages was less mature than those held 72 hours at 70 F prior to storage at the holding temperature of 40 F. Concurrent tests however, showed that less mature fruits were more sensitive to chilling (Table 21). Preconditioning made the fruit more resistant (Table 21 and Fig. 18); thus, the conditioning effect more than offset the influence of maturity.

TABLE 20. Effect of temperature preconditioning on the incidence of chilling injury in 'Persian' limes and 'Duncan' grapefruit.

Treatment[a]	Fruit pitted[b] Limes (%)	Grapefruit (%)
70 → 65 → 60 → 55 50 → 45 → 40 F (Daily intervals)	25.6	53.1
70 → 60 → 50 → 40 F (2 day intervals)	33.2	39.8
70 → 55 → 40 F (4 day intervals)	28.1	45.5
70 → 40 F (After 8 days)	31.9	57.0

[a]Fruits were transferred among cabinets maintained at specified temperatures.

[b]Limes observed after 2.5 weeks at 40 F. Grapefruit observed after 6 weeks at 40 F.

TABLE 21. Effect of temperature preconditioning on the incidence of chilling injury in 'Lacatan' bananas.

Treatments[a]	Location on the bunch[b]	Percentage aggregate of pitting per fruit[c] With regard to location (A)	Without regard to location (B)
A.			
	I	4.8	
70 → 65 →	II	7.0	
60 → 55 →	III	9.3	
50 → 45 →	IV	8.7	8.9
40 F	V	8.7	
(12 hr intervals)	VI	10.0	
	VII	10.0	
B.			
	I	6.0	
	II	5.7	
	III	8.7	14.4
70 → 60 →	IV	10.0	
50 → 40 F	V	14.3	
(24 hr intervals)	VI	16.0	
	VII	20.0	
C.			
	I	50.0	
	II	56.7	
70 → 55 →	III	61.7	
40 F	IV	70.0	57.0
(36 hr intervals)	V	80.0	
	VI	80.0	
	VII	78.3	
D.			
	I	90.0	
	II	90.0	
70 → 40 F	III	88.3	
(In 72 hrs)	IV	91.7	90.6
	V	91.7	
	VI	91.7	
	VII	95.0	

[a] Fruits were transferred to cabinets maintained at specified temperatures.

[b] A bunch had 14 hands of 16 fingers each. I=first two stem-end hands= VII= last two blossom-end hands.

[c] Observed after 10 days at 40 F; column B is not the average of column A.

TABLE 22. Effect of temperature preconditioning on chilling sensitivity of 'Booth 5' avocado.

Treatments[a]	Fruit pitted after 4 weeks at 40 F[b]
70 → 65 → 60 → 55 → 50 → 45 → 40 F (Daily intervals)	1.7
70 → 60 → 50 → 40 F (2 day intervals)	13.3
70 → 55 → 40 F (3 day intervals)	21.7
70 → 40 F (After 5 days)	30.0

[a]Fruits were transferred to cabinets maintained at specified temperatures.

[b]Percentage of fruit surface affected in the aggregate.

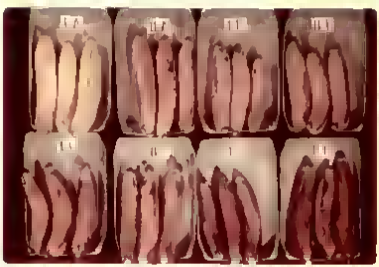

Figure 18. Effect of temperature preconditioning on the incidence of chilling injury in 'Lacatan' banana. (I - Green tip prior to temperature conditioning. II - Green, trace of yellow prior to temperature conditioning. A - temperature reduced from 70 F to 40 F in 5 F steps at 12 hour intervals; B - in 10 F steps at 24 hour intervals; C - in 15 F steps at 36 hour intervals, and D - for 30 F after 72 hours).

Partial Vacuum at Chilling Temperatures

A recent paper by Burg and Burg (34) indicated that the storage life of bananas, limes, avocados, and tomato could be greatly extended by holding fruits under partial vacuum at recommended storage temperatures. It was decided to see if reduced pressure would effect chilling at less than optimum holding temperatures. When fruits were held at 40 F and then subjected to a partial vacuum of 220 mm of mercury, occurrence of chilling injury was retarded more in limes (all sound) than in bananas, 26.9%, or in avocados, 11.1% (Table 23). Control lots in grapefruit did not develop marked pitting probably because fruits were picked late during the season (see Preharvest Factors).

Controlled Atmosphere Storage

Little research has been devoted to controlled atmosphere (CA) storage of tropical and subtropical fruits as compared to deciduous fruits (96). There are several accounts of CA storage of avocados (17, 18, 19, 20, 154, and 162), a few on lemons (22, 27, 73, and 155), and only one each on limes (158) and bananas (204). In general, benefits, such as lowered respiratory activity, delayed ripening, and retarded color change, have been reported from raising CO_2, lowering O_2 concentrations, or both (94, 95, 102, 159, 161, and 174). These findings were confirmed in the present study. Green rind color of limes

TABLE 23. Effect of partial vacuum on chilling sensitivity of banana, lime, avocado, and grapefruit.

Fruits[a]	Air pressure (mm Hg)	Fruit pitted (%)
'Lacatan' banana	220	26.9
	760	92.8
'Persian' lime	220	0
	760	65.4
'Booth 5' avocado	220	11.1
	760	31.3
'Marsh' grapefruit	220	4.4
	760	23.5

[a]Bananas and limes held for 4 weeks, avocados for 5 weeks, and grapefruit for 7 weeks, all at 40 F. Percentage of fruit surface affected in the aggregate.

was maintained at 6% O_2 concentration and the CO_2 evolved by the fruit was reduced by 5.1 mg hr^{-1} kg^{-1} from that of the control although decay was increased from 3.75 to 11.25% (Table 24).

The effect of modified atmospheres to prevent chilling injury has not been noted elsewhere. A test on the effect of CA storage on pitting was conducted on limes. Modification of storage atmosphere affected pitting. A concentration of 7% O_2 was optimal in preventing chilling injury (Fig. 19). An atmosphere of pure oxygen produced severe pitting of limes, but one with no oxygen resulted in even more.

These results (from temperature preconditioning, humidity control in storage, partial vacuum at chilling temperature, and CA storage) indicate certain modest, readily available means of reducing chilling injury. Fruits, especially bananas, received in hot weather should not be unnecessarily exposed to high ambient temperatures. Prompt cold storage favors chilling injury whereas a gradual reduction in temperature mitigates the effect. Control of humidity at near 100% can be expected to reduce susceptibility to chilling. Reduction of chilling injury in partial vacuum and at low O_2 levels with normal CO_2 concentrations may afford a useful approach to further study of physiological and biological aspects of the problem.

TABLE 24. Effect of controlled atmosphere storage on respiratory activity, rind color, and decay of 'Persian' limes held for 4 weeks at 40 F.

Criteria	Atmosphere	
	Air	6% O_2
Respiratory activity (mg CO_2 hr^{-1} kg^{-1})	0.92	0.96
Rind color[a] (Absorbance at 675 mμ)	23.25	17.15
Decay (%)	3.75	11.25

[a]High absorbance values indicate dark green. Low values, pale green or yellow.

Figure 19. Effect of storage in the presence of 6 O_2 concentrations on the incidence of chilling injury in 'Persian' limes. Atmosphere control is accurate to ca. ± 1%.

Histological Observations on Chilling Injury

Symptoms of chilling injury varied according to the type of tissue involved. Pitting was more evident with fruits, such as limes, grapefruit (Figs. 20 and 21), or avocado, in which the outermost covering is harder and thicker than the adjacent layers. Watersoaking, as in tomato, or general surface discoloration, as in banana (Figs. 22, 23, and 24), predominated when the peel is thin or about as soft as the flesh. The smoky appearance observed in chilled banana (Fig. 18) may have resulted from loss of membrane integrity in epidermal cells, since entrance of oxygen through cells was apparently enhanced (24). Tannins were oxidized and appeared as dark granular bodies (Fig. 23) which became opaque when they coalesced, thus causing the discoloration observed on the surface of the fruit. In limes and grapefruit however, cells between vessels and epidermis became constricted (Fig. 21) probably because of partial desiccation of tissue. However, it is assumed that pitting, watersoaking, or general surface discoloration are only secondary manifestations of a basic process which might be designated the chilling syndrome.

Physiological Investigations

Rind Color

Green rind color is desired for marketing of limes (117). Presently, cold storage is the only means to

Figure 20. Cross-section of the peel of normal grapefruit. Note the thickness of the flavedo tissue. x1280.

Figure 21. Cross-section of the peel of pitted grapefruit. Note the constriction of [illegible] tissue x640.

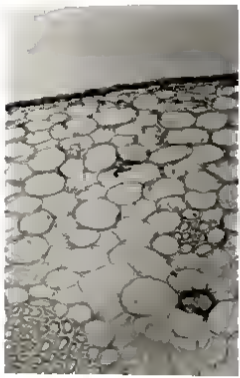

Figure 22. Cross-section of the peel of normal banana. Note the paucity of dark granular bodies. x640.

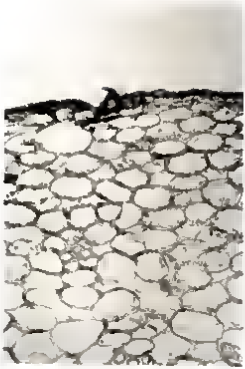

Figure 23. Cross-section of the peel of pitted banana. Note the abundance of dark granular bodies. x640.

Figure 24. Cross-section of the peel of pitted banana. Note smoky appearance and surface discoloration of cells. x1250.

retard color change. The temperature at which good color will persist, below 50 F, is on the margin of chilling (Fig. 25); hence, the color problem is inextricably linked with the chilling problem.

The change from green to yellow color on limes is related to temperature (Fig. 25 and Table 25C), humidity (62, 139), and light (139). Other factors however, may have immediate and far-reaching influences; for example, rough handling hastened yellowing, 72% absorbance at 675 mμ, (Table 25A) especially in limes picked in the morning, 64% (Table 25B). Also, a reduction from normal atmospheric pressure of 21% to 7% O_2 retarded the green to yellow color change in limes (Fig. 19). The delayed yellowing at low oxygen tensions could result from a lowered respiratory activity.

Respiratory Activity

Rough handling soon after picking in the morning and at noon, increased respiration of the fruit (Table 26). However, respiratory activity was not stimulated when rough handling followed afternoon picking or was delayed until 24 hours after picking. This effect may only be associated with turgidity changes throughout the day but as far as the writer is aware, such observations have not been reported by other workers.

Limes held at 60 F subsequent to 3 weeks' storage at 32 and 40 F had much higher CO_2 evolution than those

Figure 25. Effect of temperature on rind color and on the degree of pitting in 'Persian' limes (F).

TABLE 25. [illegible] of [illegible] with [illegible] picked, and [illegible] temperature [illegible] color of 'Persian' limes.

Treatments	Color values after 4 weeks Absorbance at 675 mμ[a] (%)
A. Handling method[b]	
Very careful	97
Normal	95
Immediate rough	72
Rough after 24 hrs	87
B. Time of day when picked[b]	
Morning — Normal	97
Morning — Rough	64
Noon — Normal	90
Noon — Rough	78
Afternoon — Normal	87
Afternoon — Rough	83
C. Holding temperature (Normal handling)	
32 F	105
40 F	92
50 F	87
60 F	79
70 F	71

[a]High absorbance values indicate dark green. Low values, pale green or yellow. Five replications of 10 fruits each.

[b]Held at 50 F.

Table [illegible]. Effect of handling methods, time of handling, and holding temperature on respiratory activity of 'Persian' limes.

Treatments		Respiratory activity[a] (mg CO_2 $hr^{-1}kg^{-1}FW$)
Handling method[b]		
Very careful		8.0
Normal		8.4
Immediate rough		10.3
Rough after 24 hrs		8.5
Time of day when picked[b]		
Morning	Normal	7.6
	Rough	9.1
Noon	Normal	9.1
	Rough	10.3
Afternoon	Normal	9.5
	Rough	8.4
Holding temperature (Normal handling)		
	32 F	31.6
	40 F	17.2
	50 F	9.5
	60 F	7.4
	70 F	7.4

[a]Determined at 60 F. subsequent to 3 weeks storage at indicated temperatures. Increase in respiration rate indicates internal injury. Five replications of 2 fruits each.

[b]Held at 50 F.

held at 50, 60, or 70 F (Table 26). Apparently, low temperature damaged the tissue and thereby increased catabolic activity. Oxygen uptake of lime tissues taken from fruits held at chilling and non-chilling temperatures was determined to see how respiration would be affected at an advanced stage of pitting. Lesions - necrotic rind tissue - from fruit that showed pitting were compared with apparently sound tissue on the same fruit. The more advanced was the degree of pitting, the lower the respiratory activity in limes previously stored at 32 F for 3 weeks (Table 27A-D). Thus, it seemed that fruits slowly deteriorated until death of the tissues finally ensued. However, the ultimate decrease in respiration rate was not unique for tissues held at lower temperatures, since 70 F also resulted in depressed metabolic activity (Table 27H), probably because of partial dessication of tissue (15, 77, 134). Fruit stored at 50 F showed a slight decrease in respiration, 148.73 μl O_2 over that held at 40 F, 129.96 μl O_2, although there was no pitting (Table 27F). It could be that at 50 F, physiological disturbance has started but pitting has not yet begun. Thus pitting could be the result of a previous physiological upset.

Respiratory activity of flavedo, albedo, and vesicles from 'Duncan' grapefruit showed that most of the O_2 uptake occurred in the flavedo both in the pitted and the slightly pitted fruit (Table 28). Pitted sections of flavedo and albedo tissues had higher respiratory activity than the unpitted portion on the same fruit.

TABLE 27. Respiratory activity of peel sections taken from 'Persian' limes subjected to chilling and non-chilling temperatures for 3 weeks.

Temperature (F)	Pitting		Oxygen uptake[a]	
	Fruit (%)	Sections (%)	Fresh weight basis ($\mu l\ O_2\ hr^{-1} g^{-1}$)	Dry weight basis
A. 32	100	100	3.27	12.62
B. 32	50	100	36.52	119.26
C. 32	50	50	59.74	232.00
D. 32	50	0	84.79	386.80
E. 40	25	25	31.01	129.96
F. 50	0	0	39.82	148.73
G. 60	0	0	49.18	201.87
H. 70	0	0	43.14	116.26

[a]Determined at 70 F 12 hours after removal at respective holding temperatures.

TABLE 28. Respiratory activity of vesicles, flavedo, and albedo sections taken from 'Duncan' grapefruit held at 40 F for 6 weeks.

Treatment	Oxygen uptake at 70 F following removal of fruits at 40 F	
	Fresh weight basis ($\mu l\ O_2\ hr^{-1} g.^{-1}$)	Dry weight basis ($\mu l\ O_2\ hr^{-1} g.^{-1}$)
Pitted fruit		
Pitted section		
Flavedo	255.6	692.6
Albedo	162.4	541.2
Vesicles	20.1	198.8
Non-pitted section		
Flavedo	174.5	608.8
Albedo	117.1	402.2
Slightly pitted fruit		
Slightly pitted section		
Flavedo	266.2	775.4
Albedo	145.5	533.3
Non-pitted section		
Flavedo	162.4	490.0
Albedo	73.9	247.6

Results in bananas corroborated those in grapefruit. Respiratory activity was confined mostly to the peel (Table 29). Respiratory activity of the flesh was more or less constant regardless of previous chilling experience. Also, O_2 uptake of sliced sections from the peel increased as the color of the fruit changed from green to yellow. The effect of an advanced degree of pitting was more spectacular in bananas than in limes. One or two days at 40 F delayed and depressed that climacteric rise of bananas removed to 70 F (Fig. 26). However, the climacteric rise approached that of the unchilled fruit as the chilling period was extended to 5 days. The climacteric ascent was absent in bananas previously chilled for 7 days.

Oxygen uptake of tissue discs taken from previously chilled avocado and tomato fruits was higher than that of unchilled ones (Table 30B). This result is consistent with those obtained for other fruits.

Membrane Permeability Studies

Oxygen uptake of tissue, as described above, was increased as a result of exposure to chilling temperatures (Table 27A-D), possibly because of a loss in membrane integrity of tissue. Evidence associating permeability and low temperature has been reported (104, 106, 108, 113, 148). However, it is not clear in the literature whether changes in permeability are an early response to chilling, unique to chilling sensitive plants, or are a general response of all

TABLE 29. Respiratory activity of flesh and peel sections of banana at various stages of maturity.

Part of fruit	Peel color[a] (index number)	Respiratory activity[b] ($\mu l\ O_2\ hr^{-1} g^{-1}$ FW)
Peel	Green (1)	96
	More green than yellow (3)	102
	Green tip (5)	111
Flesh	Green (1)	74
	More green than yellow (3)	74
	Green tip (5)	70

[a]From the Banana Ripening Manual (4).

[b]Determined at 70 F, 3 days after removal from 50 F.

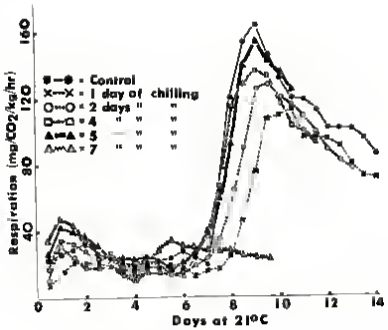

Figure 26. Respiratory activity of 'Lacatan' bananas [illegible] room after removal from various periods at

TABLE 30. Respiratory activity of peel sections and electrolytic conductivity of leachate from unchilled and chilled fruits.

Criteria	Fruits	Treatment: Unchilled	Treatment: Chilled[c]	Increment (increase in conductivity)
A. K, micromhos[a]	'Valencia' orange	56.75	61.75	5.00
	'Persian' lime	78.75	95.50	16.75
	'Lacatan' banana	98.50	134.00	35.50
	'Duncan' grapefruit	68.50	85.75	17.25
B. Respiratory activity of fruit sections[b] ($\mu l\ O_2\ hr^{-1}g^{-1}$ FW)	'Booth 5' avocado	52.25	70.00	-
	'Floradel' tomato	31.20	41.75	-

[a]Based on 100 sq cm of fruit surface.

[b]Determined at 70 F.

[c]Normal fruits held for 2 weeks at 60 F; chilled fruit at 40 F.

plants to low temperatures. Electrolytic conductivity of leachate from chilled and unchilled fruit was measured. About 3 times as many ions were released from chilling-sensitive fruit tissues, such as limes and grapefruit, as compared to that liberated from orange tissues which are relatively resistant to chilling (Table 31). Increase in conductivity of leachate from bananas, 35.50 μmhos, was more than twice that obtained for lime, 16.75 μmhos, and grapefruit, 17.25 μmhos. This observation might be expected because of obvious differences in texture of peel tissue. However, it is apparent that membranes of chilling sensitive fruit tissues become more permeable at chilling temperatures than do those of tissues resistant to chilling.

Limes and bananas were wrapped with cheese cloth wetted with 2.5% solution of streptomycin to assess further the role of cell membranes on subsequent susceptibility to chilling injury. It is noteworthy that pitting was greatest in fruits treated with streptomycin, which destroys permeability of membranes (Table 31). Pitting was increased by 15.59% in orange, 76.14% in limes, and 61.20% in bananas. Oxygen uptake of streptomycin-treated grapefruit flavedo tissue was high, 'Duncan' being more responsive than 'Marsh' (Table 31). This result could mean that streptomycin and chilling temperatures have similar effects.

Lipid Contents

Exposure to a series of conditioning temperatures makes chilling-sensitive fruits resistant to chilling. Such

TABLE 31. Influence of streptomycin on pitting and respiratory activity of fruits.

Criteria	Fruit	Treatment: Control	Treatment: Streptomycin
Fruit pitted[a] (%)	'Valencia' orange	16.62	32.19
	'Persian' lime	9.26	85.40
	'Lacaten' banana	30.00	91.20
Respiratory activity of peel sections[b] ($\mu l\ O_2\ hr^{-1}\ g^{-1}$ FW)	'Marsh' grapefruit	74.0	87.5
	'Duncan' grapefruit	64.5	96.5

[a] Observed after 6 days for banana and 4 weeks for limes and orange, all at 40 F.

[b] Determined at 70 F 2 days after picking. Streptomycin (2.5%) was placed inside the main compartment of reaction vessel together with peel sections.

preconditioning may cause variations in fatlike constituents of tissues. Several workers have considered the role of lipids to chilling sensitivity, presumably because of their classic role in contributing to membrane integrity (83, 113, 135, and 197). For example, Lyons et al. (113) compared the physical characteristics and fatty acid composition of mitochondrial membranes isolated from several chilling sensitive and non-sensitive plants. Behavior of some species was intermediate however, and thus inconsistent. Special interpretation was required to relate their response to chilling sensitivity.

Column chromatographic separation of lipids showed that the neutral lipid fraction (triglycerides) in oranges was about twice as large as it was in grapefruit (Table 32). There was no difference in the phospholipid content of tissues.

Accumulation of Metabolites

Organic acids. Increases in respiratory activity of fruits subjected to chilling temperatures could also be interpreted as resulting from a disturbance of metabolically controlled processes rather than a variation in membrane characteristics. James (87) reported that low temperature induced accumulation of organic acids in some tissues, hence it is possible that these accumulated acids or other metabolic intermediates, might account for the respiratory stimulation upon transfer to a higher temperature. It was suggested by

TABLE 32. Lipid content of unchilled and chilled grapefruit and orange.

Fruits	Treatment[a]	Lipid content ($g\ kg^{-1}$ fresh peel tissue)
'Duncan' grapefruit	Unchilled	3.25
	Chilled	2.77
'Valencia' orange	Unchilled	7.21
	Chilled	7.37

[a]Unchilled fruits held at 60 F, chilled fruits at 40 F, all for 4 weeks.

Hulme et al. (85) that organic acids which accumulate at low temperature could be succinic, oxaloacetic, α-ketoglutaric or pyruvic. Accumulation of toxic products was postulated by Platt (144). Bake (64) mentioned that the accumulation of excessive amounts of certain metabolites may result in physiological disorder and death of cells.

Two approaches were used, organic acid analysis of pitted and unpitted fruit and organic acid application to induce pitting. Organic acid analysis by paper chromatography showed that malic and quinic acids accumulated at 32 and 40 F (Table 33). This result with limes was corroborated with bananas. Green bananas (more susceptible to chilling) had higher malic and quinic acid contents in the peel as compared with fruits which were turning yellow (Table 34). Direct application of the acids to whole lime fruits held at 60 F did not lead to chilling injury, probably because the solution failed to enter the highly cutinized fruit surface. Peel sections of limes in prolonged contact with malic acid became pitted, whereas those with quinic acid did not (Fig. 27). However, α-ketoglutaric, succinic, and citric acids also caused pitting; an obscure but interesting complication. These results confirm observations by Hulme et al. in English grown apples (85) which are remarkably susceptible to low temperature breakdown.

Metabolic inhibitors. It has been shown in this study that respiratory intermediates accumulated during

TABLE 33. Organic acid content of unchilled and chilled 'Persian' limes.

Part of fruit	Holding temperature	Organic Acids[a] (10^{-2}M)						
		Fumaric	Succinic	Ketogluteric	Malic[b]	Citric	Tarteric	Quinic[c]
Peel	32 F	++	+++	+	++	++	+++	-
	40	++	+++	+	+++	++	+++	-
	50	++	++	+	+	++	+++	+
	60	++	++	+	+	++	++	++
	70	++	++	+	+	++	++	++
Juice	32 F	+	++	-	+++	+++	-	+
	40	-	++	-	+++++	+++	-	+
	50	-	++	+	+++	++	-	+
	60	-	++	-	++	++	-	++
	70	-	++	-	++	++	-	++

[a]Determined by thin-layer and column chromatography after 3 weeks at respective holding temperatures. (+++++ = very high, ++++ = high, +++ = moderate, ++ = low, + = very low, - = undetected.)

[b]Note the relatively high amount of malic acid at 40 F in the juice.

[c]Note the high amount of quinic acid at 32 to 50 F in the peel.

TABLE 34. Organic acid content in the peel and pulp of 'Lacatan' banana previously held at 40 F for 10 days.

Organic acids[a] (10^{-2}M)	Peel color[b]			Pulp color[b]		
	Green (2)	Yellowish-green (4)	Yellow (6)	Green (2)	Yellowish-green (4)	Yellow (6)
Malic	++++[c]	+++	+++	+++	+++	+++
Citric	++	+	-	+++	+++	+++
Quinic	++++	+	-	++++	++++	+++
Succinic	+++	+++	+++	-	-	-
Tartaric	-	-	-	++	+	-
∝-ketoglutaric	++	++	++	+	+	+

[a]Determined by thin-layer chromatography.

[b]Color and color values from the Banana Ripening Manual (4).

[c]++++ = high, +++ = moderate, ++ = low, + = very low, - = undetected.

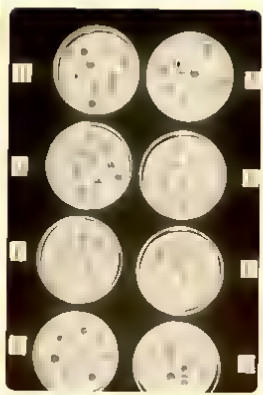

Figure 22. Effect of several substrates on [illegible] of lima bean leaves held at 60 F for 2.5 weeks (A - H_2O; B - citric acid; C - α-ketoglutaric acid; D - fumaric acid; E - succinic acid; F - malic acid; G - nicotinamide adenine dinucleotide; H - quinic acid).

chilling. Yet, it is not clear whether this effect was a consequence or a cause of the development of chilling lesions. Other workers have assumed that accumulation of metabolites could be the cause of chilling injury (64, 49, 82, 85, and 144). This conjecture was tested further by using known metabolic inhibitors at temperatures above the chilling range to interrupt the metabolic processes at known and specific points, thereby forcing the accumulation of specific intermediates. Since this did not involve chilling, production of symptoms similar to those of pitting would indicate that accumulation of these metabolites caused the lesions. Inhibiting action was observed throughout when oxygen uptake of treated grapefruit tissues was determined, except with sodium azide, a phosphorylation uncoupler and also an inhibitor of oxidases (Table 35). The most nearly complete inhibition over a 4-hour period was obtained with DNP, a phosphorylation uncoupler and an inhibitor of the Krebs cycle, which blocks α-ketoglutarate conversion to succinate. Oxygen uptake in tissues treated with inhibitors of the glycolytic pathway such as fluoride which blocks enolase activity and iodoacetate which blocks phosphoglyceraldehyde dehydrogenase activity, was lower. However, oxygen uptake in tissues treated with cyanide, inhibitor of the terminal oxidase system, was relatively inconsequential. Thus, it appears that inhibition of oxygen uptake by metabolic inhibitors was most active when the inhibitors were specific for some reaction during or before the completion of the tricarboxylic acid cycle. Typical

TABLE 35. Effect of metabolic inhibitors on respiratory activity of grapefruit.[a]

Treatment	Respiratory activity of sections at 70 F ($\mu l\ O_2\ hr^{-1}g^{-1}$ FW) Hours after treatment 1	4	Inhibition after 4 hours (%)
Control (H_2O)	97.0	105.2	-
Na Iodoacetate ($10^{-1}M$)	39.2	25.6	75.6
Na Fluoride ($10^{-2}M$)	76.4	54.6	38.6
Na Malonate ($10^{-2}M$)	107.0	97.0	7.8
2-4, Dinitrophenol ($10^{-2}M$)	54.5	22.2	78.8
Potassium Cyanide ($10^{-2}M$)	63.0	83.5	11.1
Na Azide ($10^{-2}M$)	134.1	110.2	-

[a] 'Marsh' grapefruit previously held at 60 F for 2 days.

lesions were not observed when tissues were treated with these inhibitors. The possibility of an osmotic effect superimposing on what otherwise might have developed into a typical chilling lesion was discounted because optimum osmotic concentrations of these inhibitors were established in preliminary studies. Thus, the accumulation of metabolic intermediates is apparently a result, not a cause of chilling injury.

Mitochondrial and Oxidative Phosphorylation Activity

A few obvious, but nevertheless unavoidable, limitations are incumbent upon the use of tissue discs during such investigations of metabolic processes as inhibitor experiments and organic acid analysis. One is that response of tissues, in terms of oxygen uptake, to applications of chemicals was not immediate (an effect much desired to eliminate the possibility of secondary influences that might occur within the time lag). Perhaps, this slow motion resulted from the cutinized cell wall that served as barrier to entrance of chemicals to the side of action in the mitochondria. Another limitation to the use of tissue discs was that the amount of mitochondria present in the tissue is sparse (87). These two factors may account for the low sensitivity of tissues to the application of chemicals.

Various organic acids together with the required cofactors were added to mitochondria to study the effect of excess intermediaries as a possible cause of chilling

injury. Oxygen intake of the reaction mixtures was then determined. It is known (3, 25, 86) that exogenous addition of a high amount of organic acid can decrease oxygen uptake when compared with those treated with optimal concentrations. Here, excess succinate, malate, and α-ketoglutarate inhibited oxygen uptake of grapefruit and lime mitochondria (Table 36). Inhibition by malate in limes and grapefruit, 13.36 and 20.5 μl O_2, respectively, was slightly greater than by either succinate, 9.12 and 19.7 μl O_2, and α-ketoglutarate, 7.9 and 13.9 μl O_2. Grapefruit mitochondria were markedly higher in activity than mitochondria isolated from limes. These results on organic acid inhibition were in agreement with those obtained by organic acid analysis and chemical application to tissue. However, these observations did not contribute to an understanding of the basic nature of chilling injury.

A more important effect was that obtained for oxidative phosphorylation activity of tissues. It is obvious that if P/O ratio is high, more phosphorus is consumed per unit of oxygen and therefore incorporation of phosphorus with A^{32} to form the high energy compound, ATP, is efficient. Chilled fruits may have a depressed capacity to utilize phosphorus efficiently, thus the proper amount of energy necessary for the normal operation of the various metabolic processes could not be supplied. When mitochondria from chilled and unchilled grapefruit were isolated and the P/O ratio was determined, those obtained from fruit held at

TABLE 36. Activity of mitochondrial fractions isolated fro[illegible] grapefruit in the presence of an excess of Krebs c[illegible]

Substrate (as sodium salt)	Mitochondrial Ac[illegible] (µl O_2 hr^{-1} g^{-1} f)					
	Limes					
	10^{-2}M	10^{-1}M	Increment	10^{-2}M	[illegible]	[illegible]
No substrate	0.60	0.85	+ 0.25	1.0	1.2	[illegible]
α-ketoglutarate	30.00	22.10	- 7.90	225.4	209.5	[illegible]
Citrate	22.60	20.04	- 1.56	142.3	141.0	[illegible]
Succinate	49.12	40 00	- 9.12	316.0	293.3	[illegible]
Malate	45.66	32.30	-13.36	205.7	185.0	[illegible]
Pyruvate	13.21	14.45	+12.4	134.6	138.9	[illegible]

[a]Limes previously held at 50 F for 7 days. Grapefruit previously held at 60 F for 2 days

[b]Determined at 70 F following removal from holding temperatures.

[illegible] had a T/[illegible] ratio of [illegible], whereas those held at 40 F had a ratio of only 0.71 to 0.95 (Table 37). Thus, the hypothesis is now offered that the energy-building system of the fruit is impaired by exposure to chilling temperatures.

Fruit Volatiles

Energy per se may not be directly involved in the subsequent development of visual symptoms of injury if it is true that depletion of energy is the earliest effect of chilling. Secondary effects are expected to develop in the course of tissue weakening. One such effect could be the production of by-products as a result of incomplete oxidation of metabolites which in turn occurred because of a decrease in energy supply. Low molecular weight fragments released could easily escape from cell membranes which become more permeable in the course of tissue weakening. Thus, undesirable volatile components may accumulate on or just the surface of the fruit and produce the so-called "pitting effect". This possibility was substantiated by the previous observation that "waxing" with Flavorseal 93 aggravated pitting (Table 17), whereas subjection of fruits to partial vacuum ameliorated chilling injury (Table 24). It could very well be that a volatile compound accumulates inside "waxed" fruits which then causes pitting. If this is true, partial vacuum could be expected to reduce the occurrence of pitting.

The particular type of compounds that might have

[illegible] chondrial fraction isolated from 'Marsh' grapefruit held at 60 and 40 F for 6 weeks.

Temperature	Substrate as sodium salt (10^{-2}M)	Oxidative phosphorylation (P/O ratio)	Mitochondrial activity ($\mu l\ O_2\ hr^{-1}g^{-1}FW$)
60 F	Tris-mannitol	-	10.6
	α-ketoglutarate	1.42	101.0
	Citrate	1.29	114.1
	Succinate	1.20	243.1
	Malate	1.40	154.8
	Pyruvate	1.26	109.3
40 F	Tris-mannitol	-	12.5
	α- ketoglutarate	0.95	108.3
	Citrate	0.92	120.0
	Succinate	0.71	220.2
	Malate	0.83	163.1
	Pyruvate	0.92	93.5

accumulated during storage by gas chromatography. Acetaldehyde was higher in chilled fruit than in unchilled ones (Table 38). Moreover, scald resulted when limes were wrapped with cheesecloth soaked in acetaldehyde solution. It is not understood whether the scald observed was an advanced degree of pitting or not. Perhaps, these two symptoms overlap since Brooks and McCulloch (30) commented that pitting of citrus fruits might be related to scald of apples. It was observed that pitting was reduced by 48.7% when diphenylamine was added to lime sections treated with 1.25% acetaldehyde solution (Table 39). The fact that diphenylamine can prevent both scald and pitting indicates that the two symptoms may be related. On this evidence, the hypothesis is advanced that pitting is caused by an accumulation of volatiles which in turn is attributable to low energy supply.

Table 21. Acetaldehyde content of unchilled and chilled grapefruit and orange.

Fruits	Treatment[a]	Acetaldehyde[b] (g kg^{-1} fresh fruit)
'Duncan' grapefruit	Unchilled	0.0298
	Chilled	0.2144
'Valencia' orange	Unchilled	0.0461
	Chilled	0.0532

[a]Unchilled fruits held at 60 F, chilled fruits at 40 F, all for 6 weeks.

[b]Determined by gas chromatography.

TABLE 39. Antagonistic action of diphenylamine (DFA) over acetaldehyde in pitting of lime sections.

Acetaldehyde concentration (%)	Pitting after 10 days at 40 F		Decrease in pitting (%)
	Acetaldehyde (%)	Acetaldehyde and 1500 ppm DFA (%)	
Control	10.2	12.3	-
0.07	34.5	30.6	11.2
0.15	41.0	28.5	30.3
0.31	49.3	33.4	32.2
1.25	78.4	40.3	48.7

SUMMARY AND CONCLUSIONS

Oleocellosis

[illegible] climatic and non-climatic influences were shown to affect oleocellosis in limes. High temperature with a corresponding decrease in relative humidity at about mid-day resulted in an increase in the RORP which was inversely related to oleocellosis. Also, RORP is highest for fruits on the east side and lowest on the north side of lime trees. Young limes, especially those with a rough surface, were more susceptible to oleocellosis than mature ones with smooth surfaces.

Styler-end Breakdown

Striking the apex of fruit immediately after picking produced 5 to 6 times the amount of SEB in the morning picked limes as compared with those picked in the late afternoon. This damage caused release of cell contents at the styler-tip which can be transmitted from SEB-effected fruit to sound ones. It is apparent however, that a certain degree of maturity is necessary before deliberate mechanical injury can be made to induce SEB.

Chilling Injury

Chilling injury is a physiological disorder which may

effect many fruits, particularly those of tropical or sub-tropical origin, on exposure to temperatures several degrees below 45 F either before or after harvest. Empirical observations and metabolic considerations to elucidate the mechanism of chilling injury were made.

Empirical Observations

Factors shown to affect the development of chilling symptoms were as follows:

(a) Maturity. Mature limes and grapefruit were less susceptible than less mature fruits.

(b) Position on tree. Grapefruit, both 'Marsh' and 'Duncan', were least susceptible from top of the tree as compared with fruits from lower down.

(c) "Waxing". Surface pitting of limes at chilling temperature increased as the number of "wax" applications was increased.

(d) Chemical treatments. A concentration of 10 ppm kinetin combined with 250 ppm diphenylamine markedly decreased chilling injury symptoms in limes.

(e) Temperature. Temperatures above 90 F and relative humidities below 50% caused symptoms similar to chilling injury in 'Persian' limes and 'Lacatan' bananas.

(f) Humidity. Relative humidity close to 100% prevented chilling injury symptoms.

(g) Temperature preconditioning. Gradual lowering of the temperature delayed the development of pitting in bananas

and avocado, but such effect was not observed in limes and grapefruit.

(i) Partial vacuum. A partial vacuum of 220 mm of mercury retarded the development chilling injury.

(j) Controlled atmosphere storage. A concentration of 7% O_2 was optimal to prevent chilling injury in limes and zero oxygen was more detrimental than storage in pure oxygen.

Theoretical Aspects

Recently, Eaks (61) and Eaks and Mathias (62) proposed that certain metabolic intermediate compounds, specifically organic acids, may accumulate during the chilling exposure causing irreversible physiological damage to the plant cells. In part, this finding agrees with the present study. Malic acid was shown to accumulate in limes at 40 F but not at 60 F. It is assumed that the accumulation of metabolic compounds is only a secondary effect of a more basic process. The fundamental mechanism associated with chilling injury may proceed as follows:

A progressive decline in the capacity of the fruit for oxidative phosphorylation occurs with exposure to low temperature. Utilization of phosphorus is inefficient. This could lead to a shortage of high energy compounds, typically ATP, needed for the maintenance of cell organization in the presence of enzymatic processes constantly tending to disrupt the system. A net breakdown of complex cellular components follows because of the resultant shortage of energy. The

[illegible] of chilling would explain the observed increase in [illegible] permeability, susceptibility to decay, accumulation of metabolites, and increase in oxygen uptake. Associated with ATP shortage, rind pitting (the most common symptom of chilling injury) could result from an accumulation of toxic volatiles under the cuticle which were released across permeable membranes. These volatiles, mainly acetaldehyde, may originate from sub-oxidation of substrates as a consequence of low energy supply.

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BIOGRAPHICAL SKETCH

The author, Ernesto B. Pantastico, was born in Pansol, Calamba, Laguna, Philippines, on April 21, 1938. He received his secondary education from the Laguna Institute, Calamba, Laguna, and graduated (with honors) in May, 1955. He attended the University of the Philippines and was granted the degree of Bachelor of Science in Agriculture (Honor's Curriculum) in April, 1959. He was employed for one year (1959-60) by the Bureau of Plant Industry as a research assistant and editor of a research journal. In October, 1960, he accepted a position as instructor in plant physiology in the Department of Botany at Los Baños. In 1962, he was granted a graduate research fellowship from the University of the Philippines to pursue graduate work. He received the degree of Master of Science in Botany (Plant Physiology) in May, 1964 and continued his work in the same department as an instructor.

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This dissertation was prepared under the direction of the Chairman and co-chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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